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7/25/68

THE CHEMISTRY OF ANTIBIOTIC X-5108

A THESIS

Presented to

The Faculty of the Division of Graduate

Studies and Research

by

Anne Louise Lovett

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

in the School of Chemistry

Georgia Institute of Technology

May, 1971

THE CHEMISTRY OF ANTIBIOTIC X-5108

Approved:

Chairman

Date approved by Chairman: May 27, 1971

To the M-66.

Curiouser and curiouser! cried *Alice* . . .

—Lewis Carroll
Alice's Adventures in Wonderland
Frederick A. Stokes Company
(New York, 1867)

ACKNOWLEDGMENTS

The author would like to thank Dr. John R. Dyer, not only for the opportunity to do research on this highly interesting problem, but for all his help and interest during the period of this sometimes traumatic research and the writing of this dissertation. The author is also grateful to Dr. Drury S. Caine and Dr. Charles L. Liotta who read this dissertation. Financial assistance from the National Institutes of Health and the School of Chemistry is very much appreciated.

A generous supply of Antibiotic X-5108 from Dr. Julius Berger of Hoffman-LaRoche Inc. was of great help. To Dr. C. C. Sweeley, the author is grateful for some of the mass spectra and for helpful advice.

This research would hardly have been possible without the help of those associates--Fred L. Suddath, Phillip A. Torline, and others--who not only provided muscle power when necessary, but also willingly instructed in the mysteries of nuts and bolts. For all his contributions, including the some 300 mass spectra and 100 MHz nmr spectra obtained during the course of this research, the author thanks George S. Turner.

Finally, the author especially appreciates the patience, understanding, and support of her parents and her husband during this research and the writing of this dissertation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

GLOSSARY OF ABBREVIATIONS

p.o.	per os (orally)
tlc	thin-layer chromatography
nP-A-W	tlc system, n-pentanol-acetic acid-water
E-tBuOH	tlc system, diethyl ether-*t*-butyl alcohol
B-A-W	tlc system, 1-butanol-acetic acid-water
tlc-MS	thin-layer chromatography followed by mass spectrometry
MS	mass spectrometry
GC	gas chromatography
IT	injection temperature
CT	column temperature
lpr	linear programming rate
t_r	retention time(s): all retention times are given in minutes
T_R	retention temperature(s)
OV-17	phenyl silicone stationary phase for gas chromatography
SE-30	methyl silicone stationary phase for gas chromatography
GC-MS	combined gas chromatography-mass spectrometry
HMDS	hexamethyldisilazane
TMS	tetramethylsilane *or* trimethylsilyl
nmr	nuclear magnetic resonance
s	singlet
t	triplet
d	doublet

br	broad
DSS	sodium 2,2-dimethyl-2-silapentane-5-sulfonate
J	nmr coupling constant, given in Hz
X-d_3	deuterated compound X

SUMMARY

The antibiotic X-5108 was first isolated in 1952 by Hoffman-LaRoche Inc. Laboratories from a culture broth of the new organism *Streptomyces* sp. X-5108. The antibiotic is active against a large number of bacteria and is nontoxic to human beings. The amorphous yellow sodium salt was reported to have the formula $C_{38}H_{56}N_2O_{11}Na$.

The purpose of this research was to determine as many structural features as possible for this molecule, using spectroscopic and degradative techniques.

The antibiotic was purified by chromatographing the sodium salt over Sephadex G-15, followed by converting the purified salt to the free acid form and washing it thoroughly to remove contaminating lipid materials. From the analytical data for the purified X-5108 (H^+) a formula of $C_{41}H_{60}N_2O_{12}$ (mol wt 773) was indicated from the elemental composition data and a molecular weight determination.

The neutralization equivalent (773.5) indicated a monobasic acid. The saponification equivalent indicated that two other groups reactive to base were present. In addition, the molecule was found to contain one *O*-methyl group, two *N*-methyl groups, four or more *C*-methyl groups, and *ca.* eight active hydrogens. The antibiotic was also found to be optically active. The antibiotic X-5108 was converted to a trimethylsilyl derivative for mass spectral analysis. An ion at highest mass of *m/e* 1219 was observed, suggesting the presence of five or six hydroxyl groups.

The ultraviolet spectrum of X-5108 (H^+) indicated the presence of conjugated systems; the ir spectrum demonstrated that any carbonyl groups present must be α,β-unsaturated. The nmr spectrum exhibited *ca.* 15 H in the region of protons bound to olefinic groups. The nmr spectrum further confirmed the presence of a methoxyl group and at least four *C*-methyl groups.

Several reactions were attempted in order to degrade the antibiotic. Anhydrous methanolysis yielded no sugars. Basic hydrolysis yielded methylamine, isobutyraldehyde, acetic acid, butyric acid, and a complex mixture of nonvolatile products. Acidic hydrolysis yielded a similar mixture of nonvolatile, intractable products. Permanganate oxidation was also attempted. No simple dibasic acids (except malonic acid) were identified. GC-MS of the methyl esters of the oxidation products (a complex mixture) yielded spectra of several esters that were not identified conclusively. Pyrolysis yielded a complex mixture of *ca.* 50 components.

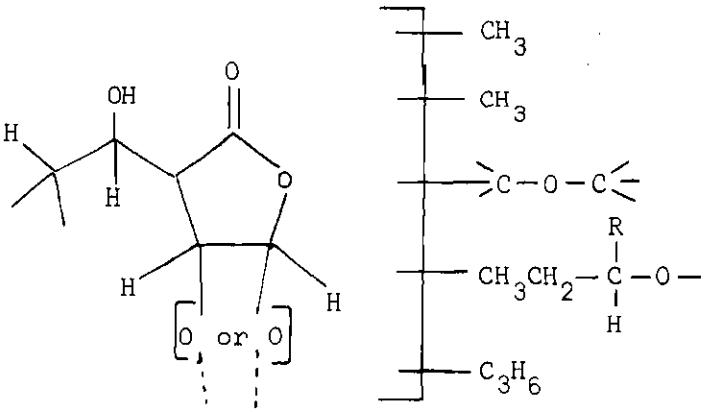
When catalytically hydrogenated, the antibiotic absorbed five moles of hydrogen per mole of X-5108. From the products of catalytic hydrogenation, a white, crystalline compound (compound X) was isolated. Elemental analysis and high resolution mass spectrometry determined the formula as $C_{16}H_{28}O_5$.

By analysis, compound X was found to contain one saponifiable group, four active hydrogens, and at least two *C*-methyl groups. The infrared spectrum indicated the presence of a γ-lactone functionality and hydroxyl groups. The ultraviolet spectrum was consistent with a γ-lactone. The compound was also found to be optically active.

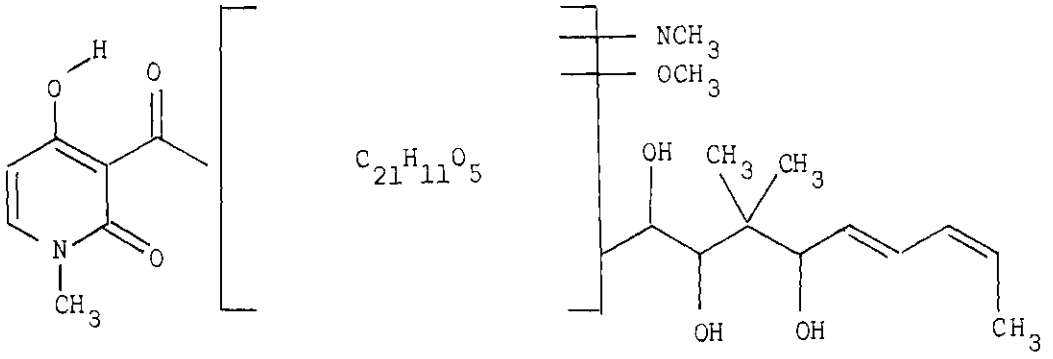
A nitric acid oxidation of compound X resulted in one major product, which was estimated to contain 12-14 carbon atoms by gas chromatographic studies. Mass spectrometry of both methyl and trimethylsilyl esters of this product revealed that no simple dicarboxylic ester was present.

When compound X was treated with concentrated hydrochloric acid, a compound with the formula $C_{16}H_{24}O_3$ was obtained (didehydro X). Its formula was confirmed by high resolution mass spectrometry. Its infrared spectrum indicated the presence of double bonds and possibly an α,β unsaturated lactone system. This latter possibility was confirmed by the ultraviolet spectrum.

The nmr spectrum of compound X, together with the other available data, indicated the part structure directly following for compound X.



It is thought that in the X-5108 molecule, two fragments isolated by Hoffmann-LaRoche Laboratories are at terminal points, and the structural moiety giving rise to compound X is located somewhere near the center. A partial structure for the antibiotic is given below.


H
O
O
N
O
CH3
C21H11O5
NCH3
OCH3
OH
CH3
CH3
OH
OH
CH3

CHAPTER I

INTRODUCTION

The antibiotic X-5108 was isolated in 1952 by Hoffman-LaRoche Inc. laboratories from a culture broth of the new organism *Streptomyces sp.* X-5108, ATCC 21386.[1]

The antibiotic shows activity *in vitro* against a number of gram-positive bacteria, such as *Bacillus simplex*, *Bacillus E*, *Sarcina lutea*, and *Streptomyces cellulosae*; it shows weaker activity against *Staphylococcus aureus*, *Mycobacterium phlei*, and *Bacillus subtilis*. It is active against the gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*, but inactive against the yeasts *Candida albicans* and *Saccharomyces cerevisiae*, and the fungi *Paecilomyces varioti* and *Penicillium digitatum*.[1]

The antibiotic is active *in vivo* against infections of hemolytic streptococci in mice at a level of 30-80 mg/kg p.o. The antibiotic is also active against pneumococci at a level of 800 mg/kg p.o. and 280 mg/kg subcutaneously. The compound is relatively nontoxic; the LD_{50} in mice is >1000 mg/kg subcutaneously and >2000 mg/kg p.o.[1]

The antibiotic was isolated from the culture medium by solvent extraction of the broth filtrate, followed by concentration of the extract and countercurrent distribution for further purification.

The sodium salt of the antibiotic was an amorphous yellow powder that decomposed at 175° after sintering at 150°. The formula suggested

was $C_{38}H_{55}N_2O_{11}Na$ (calculated: C, 61.77; H, 7.51; N, 3.79; Na, 3.11). The found analytical percentages were: C, 61.55; H, 7.95; N, 3.62; Na, 3.29 (an average of three analyses for two different samples).[1] The compound was optically active $\left[[\alpha]_D^{26} - 71° \text{ (} c \text{ 0.5, ethanol)}\right]$. The antibiotic was water-soluble, soluble in 95% ethanol and propylene glycol, but insoluble in glycerol. In a 1% aqueous solution the *p*H was 8.1, and the compound was unstable to heat in this solution. A molecular weight of about 1000-1500 was indicated by diffusion and ultracentrifuge experiments. The yellow antibiotic gave a reddish-brown coloration in methanol with aqueous ferric chloride. In methanolic hydrogen chloride, a deep green color was produced. The ultraviolet spectrum of antibiotic X-5108 in isopropyl alcohol exhibited maxima at 230 and 318 nm ($E_{1\,cm}^{1\%}$ 650 and 300, respectively), with a shoulder at 290 nm. The infrared spectrum of the compound (potassium bromide pellet) was complex, exhibiting a weak absorption at 1739 k and a much more intense absorption at ~ 1645 k, among others.

When X-5108 was treated with acids, strong bases, or oxidants, the *in vivo* activity was destroyed. In such "deactivated compounds" a new infrared absorption appeared at 1724 k and the ultraviolet absorption at 318 nm disappeared.

The free acid of X-5108, in the purest form thus far obtained (amorphous) had an optical rotation $[\alpha]_D^{26}$ - 100° (*c* = 1, ethanol). Elementary analyses for this compound (C, 64.45; H, 7.49; N, 3.80; an average of four analyses from two different samples) indicated a formula of $C_{38}H_{56}N_2O_{11}$ (Calculated: C, 63.66; H, 7.88; N, 3.91).

The compound exhibited instability, as indicated by a certain degree of photosensitivity and atypical behavior during countercurrent distribution experiments. The free acid was observed to be less stable than the sodium salt toward acids, bases, and oxidants, both in the dry form and in methanolic solution.

CHAPTER II

EXPERIMENTAL

Apparatus and Techniques

Chromatography

Column chromatography was performed using silicic acid (Mallinckrodt 2847), Unisil (Clarkson Chemical Co.), Sephadex dextran gel (Pharmacia Fine Chemicals) and Darco G-60 carbon (Atlas Powder Co.).

For general thin-layer chromatography, 0.25 mm plates of Silica Gel HF_{254} (E. Merck AG, Darmstadt, Germany) were used. These were prepared as described by Randerath.[2] Detection was accomplished by uv light, iodine vapor, or spray reagents. Specific spray reagents and unusual adsorbents are described in the text. Some spray reagents were available in aerosol cans (E. Merck).

Plates for preparative thin-layer chromatography[3] were 1.0 mm in thickness. The zones were scraped from the plates into small sintered-glass funnels, and the materials were eluted with methanol. After evaporation of the methanol, the substances were dissolved in chloroform and filtered through Whatman No. 50 hardened paper.[3]

For TLC-MS,[4] plates of 0.75 mm thickness were used. The zones were scraped from the plates with a spatula onto boats of aluminum foil. The zones were dried in air or *in vacuo*, depending upon the solvent system used. The samples were vaporized directly from the silica gel in the inlet of the mass spectrometer.

Gas chromatography was accomplished using the following gas chromatographs: a Glowall Corp. Chromalab Model A-110 (argon ionization detector) equipped with a Minneapolis Honeywell continuous recorder (50 mV full scale), a Varian Aerograph Autoprep (thermal conductivity detector) equipped with a Minneapolis Honeywell recorder (1 mV full scale), and a Varian Model 1740 (dual hydrogen flame detectors) connected to a Varian Model 20 recorder (1 mV full scale). Columns for the Glowall instrument had been prepared in these laboratories.[5] Columns for the Autoprep instrument were similarly prepared, except for a 20-foot aluminum preparative column (3/8 in. o.d., 30% SE-30) that was obtained from Varian. For routine work using the Varian Model 1740, a stainless steel column (5' × 1/8", 3% SE-30 on Varasorb) supplied by Varian was used. A 12-foot aluminum column (12' × 1/8", 3% OV-17 on acid-washed, silanized Chromosorb W) and an aluminum Porapak Q column (6' × 1/8") prepared in these laboratories were also used. Other columns are described in the text as necessary. A carrier gas flow rate of 20-30 ml/min was generally used for gas chromatography.

Spectra

Infrared spectra were determined using Perkin-Elmer Models 457 and 137 infrared spectrophotometers. A Cary Model 14 ultraviolet recording spectrophotometer was used for the ultraviolet spectra. For nmr spectra, Varian A-60A and A-60D nmr spectrometers, equipped with variable temperature probes, were used. For 100 MHz nmr spectra, a Jeolco Model 4H-100 instrument operated in the field sweep mode was used. In the frequency sweep mode, the 100 MHz instrument was used for

spin-decoupling experiments. Reference compounds for nmr spectra were TMS or DSS. Tetramethylsilane was also used as a locking signal for the Jeolco instrument.

A Varian M-66 cycloidal-focusing medium-resolution (maximum $M/\delta M \sim 5000$) mass spectrometer, interfaced with a Varian Model 200 gas chromatograph and employing a Llewellyn separator, was used for routine mass spectrometry and GC-MS. High resolution mass spectra were obtained from the High Resolution Mass Spectrometry Laboratory, Florida State University, Tallahassee, Florida. Instrumentation included an A.E.I. Picker MS 902 ultrahigh resolution mass spectrometer (maximum $M/\delta M \geq 200,000$) coupled with a Picker MSDS II mass spectrometry data reduction system.

A number of GC-mass spectra were obtained from Michigan State University and from the University of Pittsburgh through the courtesy of Dr. C. C. Sweeley. For these spectra an LKB Model 9000 single-focusing GC-MS system utilizing a molecular separator of Becker-Ryhage design was used.

All mass spectra were determined using an electron energy of 70 eV unless otherwise stated.

Standards Used for Mass Spectrometry. In all GC-MS work, standards were used. Occasionally, if it was suspected that a standard peak obscured an important peak, the spectrum was determined again with no standard present (under the same conditions).

The most common standard used was 1,1,2,2-tetrabromoethane, which has ion clusters at 79-82, 91-94, 104-107, 158-162, 171-175,

182-188, 263-265, and 342-350. For this reason, ions in these regions of the spectra are not given in the graphs and tables unless a clearly defined doublet separated by less than one mass unit was observed, or the spectrum was determined without standard.

Another standard used was perfluorotributylamine. All mass spectra determined using this standard were determined again without standard.

Miscellaneous

Methanol, benzene, cyclohexane, hexane, and dioxane were always distilled before use. Chloroform and ethanol were distilled when necessary. Pyridine was distilled and stored over sodium hydroxide pellets. Acetic acid (Fisher A-38) used for catalytic hydrogenations was not distilled. Dry methanol refers to methanol distilled from magnesium methoxide. Ether (Fisher E-138 Anhydrous) was used as received.

Organic solutions were dried with sodium sulfate or magnesium sulfate. The solutions were filtered from the drying agent by gravity filtration, after which the drying agent was washed with fresh solvent. The washings were combined with the solution.

The solvents were evaporated from solution using a Rinco rotary evaporator, Model VE-1000 A, using water aspirator vacuum. In the case of solvents with boiling points ≥ 100, an oil pump was used, along with a dry ice-acetone trap.

Microanalyses were performed by Bernhardt Laboratories (Elbach über Engelskirchen, West Germany). Melting points were obtained using a Köfler hot stage and are corrected. Optical rotations were measured

with a Bellingham and Stanley polarimeter (Model 397619). The sodium D line was used. Areas of some gas chromatographic and spectrometric peaks were measured with a Gelman Instrument Co. planimeter, Model 39231. All catalytic hydrogenations were carried out using a Parr Model 3911 pressure reaction apparatus.

Nomenclature

The expression X-5108 shall refer in a general way to the antibiotic. For clarification, the expression X-5108 (H^+) will be used to refer specifically to the compound in the free acid form, and X-5108(Na^+) will be used to denote the sodium salt.

Purification of X-5108 Using Sephadex G-15

Approximately 2 g of X-5108 (Na^+) was chromatographed over 400 g of Sephadex G-15 (eluent, distilled water) on a column 84 × 4.5 cm fitted with a coarse fritted disc at the outlet.* Fractions of *ca.* 30 ml were collected. A dark gold-colored band was eluted first, followed by a much larger band of bright yellow color. In one run, the fractions were lyophilized and weighed. The first band constituted less than 10% of the total weight. Samples of the two fractions were submitted to Hofmann-LaRoche laboratories for biological assay. (The cylinder-plate method of assay was used.) Five samples, including standards, were submitted. They were labeled: 1, X-5108 (Na^+) as received; 2 and 3,

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*It was discovered that after several runs the frit became clogged with Sephadex, and it became necessary to use a column with a base support of Pyrex wool and sea sand.

X-5108 (H^+) washed with hexane; 4, X-5108 (Na^+), a sample of the bright yellow fraction from the Sephadex column; 5, a sample of the dark gold-colored fraction from the Sephadex column. These data are shown in Table 1.

Table 1. Biological Assay of X-5108 Samples*

Concentration γ/ml	Std†	1	2	3	4	5
10	25.6	26.4	24.4	24.6	26.4	17.1
5	21.7	22.8	20.9	21.7	23.6	12.6
2.5	20.0	20.2	17.8	19.0	19.7	10.7
1.25	16.5	16.5	15.8	15.7	17.2	t2

*Dose response in mm measured against *Bacillus simplex* HLR No. 164.

†Hoffmann-LaRoche RO-2-7755/270 standard with an assigned potency of 400 units/mg.

In subsequent purifications, the following procedure was used. After Sephadex chromatography, fractions were selected that appeared to have a clear or nearly clear bright lemon-yellow color. These fractions (about 250 ml total) were combined and poured into approximately 200 ml of water acidified with hydrochloric acid to *p*H 1. While the flask was swirled, the X-5108 (H^+) precipitated as a flocculent mass.

This mixture was then extracted with 50 - 100 ml of chloroform. The chloroform solution was poured slowly into 200 ml of cold (*ca.* 5°) hexane and the mixture was either stirred for two hours or shaken thoroughly and allowed to stand overnight in the refrigerator.

The X-5108 (H^+) was then filtered using a 10 cm Büchner funnel through Whatman No. 50 hardened filter paper. The solid was then washed with several portions of hexane and spread on aluminum foil. After air-drying, the X-5108 (H^+) was then dried *in vacuo* for two hours.

From two grams of X-5108 (Na^+), 500 - 750 mg of purified X-5108 (H^+) was obtained. The material purified in this manner had a melting point of 125-127°C and did not sinter or decompose when heated to 270°.

Properties of the Dark Gold-Colored Fraction

The dark gold-colored fraction, when lyophilized, was a powder. The material did not dissolve completely in methanol.

In aqueous solution, when poured into *p*H 1 hydrochloric acid, it did not flocculate. No material was extracted by chloroform.

Nature of the Hexane-Soluble Material. The hexane used for washing each of several samples of X-5108 (H^+) was evaporated. This yielded 8-10% by weight of residue. In a methanolysis experiment done using unpurified X-5108, GC-MS had indicated that methyl esters of fatty acids were present in the volatile materials. Hence, the X-5108 hexane-soluble residue was examined for similar compounds. In a typical experiment, approximately 60 mg of the hexane-soluble material was dissolved in 5 ml of methanol, and an equal volume of 2,2-dimethoxypropane was added. One drop of concentrated hydrochloric acid was then added, and the mixture was allowed to stand for five hours at room temperature. The solvents were evaporated from the products, and the residue was dissolved in ether. The ethereal solution was washed first with an equal volume of 5% aqueous sodium bicarbonate and then with the same volume

of water. The solution was concentrated for isothermal gas chromatography. Identification of the resulting compounds was proposed by comparison of retention times with those of standards. The compounds identified were the methyl esters of lauric, myristic, pentadecanoic,* palmitic, heptadecanoic,* stearic, and arachidic acids. Methyl palmitate was the most abundant component. In addition, several other compounds were present.

It was found by the following procedure that the X-5108 (H^+) that had been purified by Sephadex chromatography and precipitation with hexane was free of fatty acid impurities. A sample of this X-5108 (H^+), 149 mg, was dissolved in 10 ml of methanol, and 20 ml of 2,2-dimethoxypropane was added. Two drops of concentrated hydrochloric acid and 0.5 ml of dimethylsulfoxide were also added. The mixture was then stirred for five hours. After removal of the solvents, a methanol solution of the residue obtained was injected into the gas chromatograph (6' 3% SE-30 column; CT 200°). No compounds were observed to be eluted.

A similar experiment with X-5108 (Na^+) that had been converted to the free acid X-5108 (H^+) and stirred with hexane indicated by the GC record that a small amount of methyl palmitate (identified by retention time) remained.

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*Concluded by interpolation using a semi-logarithmic plot of log retention time *vs* carbon number for the even-carbon acids.

Properties of X-5108

Thin-Layer Chromatography. Unpurified, hexane-washed X-5108 (H^+) exhibited three spots in the solvent system 1-butanol-acetic acid-water (4:1:5) : R_F 0.45, 0.62, and 0.83. A sample of the bright yellow fraction from the Sephadex column had only one spot at R_F 0.65. In the system chloroform-methanol (45:5) this purified X-5108 (H^+) gave two spots (R_F 0.24 and 0.35). When each of these spots were scraped from the plate, eluted, re-spotted, and developed in the same chloroform-methanol system, two spots at the same R_F values described were observed.

Analytical Data. Elemental analyses of a sample of highly purified X-5108 (H^+) were obtained. These results are presented in Table 2.

Spectral Properties of X-5108. The infrared spectrum of X-5108 (H^+), potassium bromide pellet (Plate 1), exhibited absorptions at 3400 (br), 2970, 2930, and 2880 k; strong, broad absorptions were observed in the region 1670-1520 k. The ultraviolet spectrum exhibited λ_{max}^{EtOH} 232 nm, log ϵ 4.79; λ_{max}^{EtOH} 320 nm (285 nm sh), log ϵ 4.41. The 60 MHz nmr spectra of X-5108 (H^+) in deuterochloroform (Plate 2) and X-5108 (Na^+) in a 1:3 mixture of acetone-d_6 and deuterium oxide were determined both at 35° and at 80°. Absorptions from X-5108 (H^+) occurred at τ 9.1 (*ca.* 12 H), 8.31 and 8.2 (*ca.* 8 H), 7.95 (*ca.* 4 H), 6.82 (*ca.* 5 H), 6.53 (*ca.* 5 H), 6.25 (*ca.* 3 H), 5.5-6.0 (br abs, *ca.* 7 H), 3.4-4.6 (*ca.* 15 H), and at 2.55 (d, *ca.* 1 H, J = 7).* When the temperature of this solution was

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*The number of protons was estimated by two methods: planimetry and the weighing of peaks cut from an nmr spectrum. The values given here were estimated from three weighings and one area measurement.

Table 2. Analytical Data for X-5108 (H^+)

	Value Found	Calculated for $C_{38}H_{56}N_2O_{11}$	Calculated for $C_{40}H_{58}N_2O_{12}$	Calculated for $C_{41}H_{60}N_2O_{12}$
C	63.54%	63.67	63.31	63.71
H	7.77%	7.87	7.70	7.82
N	3.58%	3.91	3.69	3.62
O(1)	25.14%	24.55	25.30	24.83
Molecular Weight (Osmometric in Benzene)	770,775	716	759	773
Neutralization Equivalent	773,774	716	759	773
Saponification Equivalent	261,262	238(2)	253(2)	258(2)
Active Hydrogen	0.98, 1.01%	1.13(3)	1.06(3)	1.04(3)
$-O-CH_3$	4.18%	4.33(4)	4.08(4)	4.01(4)
$-\underset{\vert}{N}-CH_3$	8.31%	8.10(5)	7.64(5)	7.50(5)
$-\underset{\vert}{\overset{\vert}{C}}-CH_3$	7.05%	8.37(6)	7.91(6)	7.76(6)

(1) By difference.

(2) Calculated for three saponifiable groups.

(3) Calculated for eight active hydrogens (Zerewitinoff method).

(4) Calculated for one $O-CH_3$ group.

(5) Calculated for two $N-CH_3$ groups.

(6) Calculated for four $C-CH_3$ groups.

increased, the only discernible change was in the appearance of the complexity in the region τ 3.4-4.6.

X-5108 (Na^+) was prepared for the 60 MHz nmr spectrum by converting a deuterium oxide—acetone-d_6 solution of X-5108 (H^+) to the sodium salt with solid sodium carbonate. There was a slight difference in the nmr spectra of X-5108 (H^+) and X-5108 (Na^+) in the region τ 3.4-4.6 at room temperature. When X-5108 (Na^+) was heated to 80°, the spectrum exhibited no discernible change from that at room temperature.

For the mass spectrum, purified X-5108 (H^+) was treated with HMDS and trimethylchlorosilane. A sample of purified X-5108 (H^+), 17 mg, was dissolved in 1.0 ml of dry pyridine. To this solution 2.0 ml of HMDS and 1.0 ml of trimethylchlorosilane were added. The mixture was allowed to stand at room temperature for 3.5 hr. The solvents were then evaporated, and four successive 10-ml portions of carbon tetrachloride were evaporated from the residue. The residue was triturated with hexane, and the mixture was filtered. Evaporation of the hexane filtrate gave a solid material, mp *ca.* 79°. The mass spectrum of the hexane-soluble product exhibited peaks above *m/e* 1100, but the correct masses could not be determined with certainty because of the limitations of the instrument. The highest peak observed was at *m/e ca.* 1219.

A sample of purified X-5108 (H^+) was placed directly into the inlet system of the mass spectrometer at 170° to ascertain whether any ions of interest resulting from thermal fragmentation of the molecule might be observed. Among the ions observed were: *m/e* (relative intensity) 97 (100), 85 (52.0), 107 (48.6), 43 (46.0), 296 (44.0), 41 (43.0),

55 (38.0), 278 (37.5), 152 (37.0), 81 (34.0), 122 (32.2), 182 (31.8), and 44 (28.7). An ion at *m/e* 387 (<1%) was the highest observed mass. The exact masses of the peaks at *m/e* 278 and *m/e* 296 were determined (M-66 instrument) by peak matching. Exact masses determined were 278.147 (±0.006), and 296.156 (±0.006).

Reaction of X-5108 (H^+) with Rubidium (I)

Amberlite resin IRC-50 (H^+) was converted to the rubidium phase by stirring 30 ml of the resin with 300 ml of 0.58 *M* rubidium carbonate for two hours. The resin was then washed with water until the effluent was no longer cloudy and had a *p*H of 8-9. The IRC-50 (Rb^+) was then poured into a column and washed several times with a 1:1 solution of methanol-water. Over this column was percolated a solution of 500 mg of highly purified X-5108 (H^+) in *ca.* 100 ml of a solution of methanol-water, which had been prepared by dissolving the X-5108 (H^+) in methanol, adding water to the point of turbidity, and just clearing the turbidity by the dropwise addition of methanol. The resulting solution had a methanol:water ratio of *ca.* 1:1.

The column was then rinsed with 100 ml of a 1:1 methanol-water solution. Methanol was removed from the eluate by rotary evaporation; the remaining water (*ca.* 100 ml) was removed by lyophilization. An nmr spectrum of the product (mp 163-165°) was obtained; the spectrum demonstrated only very broad absorptions.

Several solvent systems were used in attempts to crystallize the product; no crystals were obtained.

Anhydrous Methanolysis of X-5108 (H^+)

A solution of 0.107 g of hexane-washed X-5108 (H^+) and 50 ml of 7.9% methanolic hydrogen chloride was boiled under reflux for 12 hr. After evaporation of the solvents, 5 ml of pyridine and 5 ml of acetic anhydride were added to the residue. This solution was heated for 2.5 hr (*ca.* 60°) and then allowed to stand at room temperature for 24 hr. The solvents were then evaporated, and the residue was dissolved in chloroform; this solution was washed with three equal volumes of cold (*ca.* 5°) 1 *N* hydrochloric acid and three equal volumes of 5% sodium hydroxide.

The solvent was evaporated from the chloroform layer, and the residue was triturated with benzene. The benzene-insoluble fraction was dissolved in ethanol.

Gas chromatography of both fractions at 250° CT (30 min) and at 200° CT (45 min) revealed no volatile compounds. At 250°, octaacetyl sucrose (standard prepared according to Staněk and Černá,[8] mp 87.0-88.0°, lit.[8] mp 89-93°) had a t_R of 22.6 min; the t_R of methyl 2,3,4,6-tetracetyl-α-*D*-glucopyranoside (similarly prepared, mp 100-101°, lit.[9] mp 100-101°) was 1.2 min. At 200°, this standard had a t_R of 12.2 min.

Basic Hydrolysis of X-5108 (Na^+)

A solution of 0.505 g of X-5108 (Na^+) in 50 ml of 6 *N* sodium hydroxide was boiled under reflux for 24 hr. Dry nitrogen was bubbled continuously through the reaction mixture during the reaction. The mixture was then distilled into 100 ml of 6 *N* hydrochloric acid. Fifty milliliters of water was added to the reaction flask and distillation

was continued to dryness. The distillate was evaporated on a rotary evaporator (dry ice—acetone trap, vacuum pump). The solid residue was washed from the flask with 2 ml of methanol and spotted on a tlc plate. In B-A-W (4:1:5) one spot (R_F = 0.13) was observed (detection, ninhydrin). For a standard, ethylamine hydrochloride was prepared by bubbling ethylamine through 6 *N* hydrochloric acid; the excess acid was then evaporated. When tested by tlc, the ethylamine hydrochloride gave an R_F value of 0.20 in B-A-W (4:1:5). In the same system, an R_F value of 0.14 was observed for methylamine hydrochloride.

Acidic Hydrolysis of X-5108 (Na^+)

A solution of 0.50 g of X-5108 (Na^+), as received, in 50 ml of 2 *N* sulfuric acid was boiled under reflux for 19.5 hr. The reaction mixture was then adjusted to *p*H 2 with 6 *N* sodium hydroxide and distilled to near dryness. Fifty milliliters of distilled water was then added, and the distillation was continued to dryness. The *p*H of the distillate was adjusted to 10.5 and the water was evaporated; the residue was taken up in *ca.* 2 ml of water and the *p*H was adjusted to 6.4. To this solution 10 ml of ethanol and 130 mg (0.473 mmole) of *p*-phenylphenacyl bromide were added. The mixture was boiled under reflux for two hours, and when cool was extracted with three 15-ml portions of chloroform. After evaporation of the chloroform, the white residue was dissolved in benzene for gas chromatography. At a column temperature of 205° (6' 3% SE-30), no compounds were observed (the recorder was run for 40 min). Under the same conditions, standard (*p*-phenylphenacyl

acetate, propionate, isobutyrate, and butyrate) had retention times of 16.8, 23.1, 27.6, and 32.4, respectively.

The residue remaining in the distillation flask was dissolved in 50 ml of water and the *p*H adjusted to 10.5. Distillation was resumed as before. The distillate was collected in a flask containing 100 ml of 6 *N* hydrochloric acid. The solvents were evaporated and the residue was washed with methanol; the methanolic solution was tested by tlc (detection, ninhydrin solution). The results are given in Table 3.

Table 3. Thin-Layer Chromatography of Methylamine and Volatile Basic Product(s) from Acidic Hydrolysis

Solvent	B-A-W(4:1:5)	70% Ethanol	nP-A-W (4:1:5)
Plates	Silica Gel HF_{254}	Silica Gel HF 254, 0.2 M Phosphate Buffer	Cellulose
R_F Value of Methylamine	0.15	0.00-0.15	0.06
R_F Value of Sample	0.15	0.00-0.20	0.06
Literature Reference[10] R_F Value of Methylamine	0.10	0.10	0.12

Acidic Hydrolysis of X-5108 (H^+)

Hexane-washed X-5108 (H^+), 0.520 g, was dissolved in 25 ml of 2 *N* sulfuric acid and 25 ml of dioxane. After the solution was boiled under reflux for 20 hr, the reaction mixture was distilled under aspirator vacuum to remove dioxane. The remaining solution was neutralized (*p*H 6.8-7.0) with solid potassium carbonate. It was necessary to add

ca. 10 ml of water. The liquid in the flask was then decanted from the white, granular precipitate (assumed to be potassium sulfate) and placed in a continuous extraction apparatus with chloroform. After three days of extraction, the chloroform was evaporated, yielding a yellow-brown gum. This material was triturated with benzene for tlc. The benzene-soluble fraction gave R_F values of 0.11, 0.37, 0.79 (254 nm uv), and 0.66 (yellow fluorescence, 356 nm uv) in B-A-W (4:1:5). In the system chloroform—methanol (45:5), R_F values of 0.05, 0.14, 0.18, 0.39, 0.70 (254 nm uv), and 0.52 (356 nm uv) were observed.

The benzene-insoluble material was dissolved in methanol for tlc. In the system chloroform-methanol (45:5) an R_F value of 0.0 was observed. In benzene-dioxane-acetic acid (90:25:4), spots at R_F values of 0.0 and 0.28 were observed. The sample gave R_F values of 0.0 and 0.15 in benzene-methanol, and in the system ethanol-water (4:1), spots at R_F values of 0.0, 0.40, 0.50, 0.60, 0.70, and 0.75 were observed. Detection was by 254 nm ultraviolet and iodine vapor. All spots observed were also fluorescent under 356 nm light.

The benzene-soluble material was injected into the GC at 175° (6' 3% SE-30). Even though a concentrated solution was injected, the heights of the peaks observed were <5% of the solvent peak height. The peaks were observed at retention times of 0.7, 2.5, 4.3, 5.3, and 6.0. At 200° CT, peaks were observed at t_R 1.4, 4.1, and 5.5.

The solution remaining after extraction by chloroform of the acidic hydrolysis product was acidified to *p*H 1 with 6 *N* sulfuric acid and continuously extracted with chloroform for one day, yielding a

brown gum as before. This material exhibited tlc and GC behavior that was similar to that of the product obtained from extraction of the neutral solution.

Basic Hydrolysis of X-5108 (H^+)

Five hundred milligrams of hexane-washed X-5108 (H^+) was dissolved in 50 ml of 0.1 *N* sodium hydroxide and the solution was boiled gently under reflux for two hours under dry nitrogen. A gas trap consisting of an inverted funnel over a dish of freshly prepared 2,4-dinitrophenylhydrazone reagent[11] was attached to the apparatus.

Long yellow needles (0.021 g, mp 187°) were collected in the trap. The mp (lit.)[12] of an authentic sample of isobutyraldehyde 2,4-dinitrophenylhydrazone was reported to be 187°. The nmr spectrum demonstrated a doublet at τ 8.78 (J = 7 Hz), a septet centered at 7.29, and several absorptions in the region 1.0-3.0 due to the protons from the 2,4-dinitrophenylhydrazyl residue.

The reaction mixture was made neutral (*p*H 6.8-7.0) with dilute sulfuric acid and continuously extracted for 20 hr with chloroform. The chloroform was evaporated from the extract and the residue (85 mg of a yellow gum) was dissolved in methanol for GC. At 80° CT (6' 3% SE-30), retention times were 0.8, 1.9, 2.2, 5.4, and 11.5. At higher temperatures, peaks were not observed.

A basic hydrolysis was also carried out using highly purified X-5108 (H^+). A solution of 77 mg of X-5108 (H^+) in 10 ml of 6 *N* sodium hydroxide was boiled under reflux (as described previously) for 20 hr. The resulting solution was orange, with a water-soluble black scum

floating in it. The reaction mixture was then vacuum distilled to near dryness. The receiving flask, cooled with ice, contained 20 ml of 6 *N* hydrochloric acid. The distillate was then evaporated, and the product was dissolved in *ca.* 2 ml of methanol. In the thin layer system B-A-W (4:1:5), an R_F value of 0.15 was observed (ninhydrin positive). Attempts to obtain a mass spectrum of the product were unsuccessful.

The mixture in the distilling flask was then acidified with 6 *N* hydrochloric acid to *p*H 1.5, after which it was distilled to dryness. The distillate was then made basic to *p*H 11 with 20% sodium hydroxide, and the solvents were evaporated. The residue was dissolved in 2 ml of water. The *p*H was adjusted to 5.0-5.5, then 144 mg of *p*-phenylphenacyl bromide and 10 ml of ethanol were added. The mixture was boiled under reflux for 2 hr. After cooling, the reaction mixture was diluted with 15 ml of water and extracted with three 15-ml portions of chloroform. The solution was dried, and the solvent was evaporated to yield a yellow powder weighing 82 mg. Gas chromatography of the product resulted in peaks at t_R 2.0, 4.1, 5.6 (relative area 12.5) and 9.7 (relative area 1); a blank reaction gave a product with peaks at t_R 2.1 and 4.2; the standards *p*-phenylphenacyl acetate, propionate, isobutyrate, and butyrate had t_R 5.5, 7.3, 8.2, and 9.6 min; a second injection of the distillation product resulted in peaks at t_R 2.0, 4.5, 5.8 (relative area 8.6) and 9.5 (relative area 1). Peaks were also observed at t_R 7.3 (trace) and 8.2 (trace).

The residue remaining in the distillation flask was mixed with 30 ml of water, but the material was not totally soluble. The *p*H was

adjusted to 1.5, and the material was extracted with three 30-ml portions of chloroform. The chloroform layer was dried, and the chloroform was evaporated, yielding 13 mg of a yellow material. This material was dissolved in 5 ml of methanol; 5 ml of 2,2-dimethoxypropane and two drops of concentrated hydrochloric acid were then added. This mixture was allowed to stand for 3 hr, then the solvents were evaporated and the residue was dissolved in *ca.* 10 ml of ether. The ethereal solution was washed once with an equal volume of 5% sodium bicarbonate and once with an equal volume of water. The organic layer was dried and the residue was dissolved in several drops of methanol for gas chromatography. A programmed run (125°-300°, 12' OV-17) at 10°/min was carried out, but only a large aggregation of unresolved peaks, which were eluted from 250° to 300°, was observed. At 220°, isothermal GC revealed only three peaks of low intensity (t_R 1.9, 2.2, and 3.6). At 210°, peaks at t_R 2.7 and 3.1 were observed, along with a strong tail of the solvent peak.

Pyrolysis of X-5108

A total of four pyrolyses of X-5108 (Na^+), as received, were carried out. For the pyrolyses, the equipment consisted of a distillation apparatus with the distilling flask heated in a Woods metal bath and the receiver cooled in a dry ice—acetone trap. In a typical experiment, 10 g of X-5108 (Na^+) was heated. At 180° charring began, and at 210° a pale yellow material, which rapidly turned brown, was observed in the condenser. The heating was continued to 400° and this temperature was maintained until no further volatile products were evolved. The products were washed from the condenser and the cold trap with ether and the

extracts were dried, and the ether was evaporated. A product weighing 30 mg was obtained, which had R_F values of 0.0 and 0.18 in the solvent system 95% ethanol—water—28% ammonium hydroxide (100:12:16); detection was accomplished by bromocresol green spray reagent. Several standard dicarboxylic acids were chromatographed in this solvent system. An R_F value of 0.18 was observed for malonic acid; other dicarboxylic acids had higher R_F values in this system.

In a second experiment, 0.090 g of highly purified X-5108 (H^+) was dispersed in 5 ml of water. Five per cent sodium bicarbonate was added dropwise until solution was effected. A 2% solution of potassium permanganate was added to the solution until the purple color persisted after the reaction mixture had been warmed gently in a water bath (*ca.* 60°) for 30 min. After this time, the purple color was discharged by the addition of sodium bisulfite, and the solution was filtered. The mat was washed thoroughly with 30-40 ml of water. The filtrate (*p*H 5.5) was adjusted to *p*H 1.5 by the addition of *ca.* 10 drops of concentrated sulfuric acid. The acidic solution was distilled using aspirator vacuum into a receiving flask cooled in ice.

The distillate was made basic (*p*H 11) with 20% sodium hydroxide. After evaporation of the solvent, the residue was dissolved in 7 ml of water and transferred to a 50-ml round-bottomed flask. The *p*H was adjusted to 5.5, and 233 mg of recrystallized *p*-phenylphenacyl bromide and 10 ml of methanol were added. The mixture was boiled under reflux for 3 hr. The mixture was cooled, diluted with 10 ml of water, and extracted with three 15-ml portions of chloroform. The chloroform

solution was dried with magnesium sulfate. The yield from the pyrolyses averaged *ca.* 600 mg from 10 g of X-5108 (Na^+).

Gas chromatography of the pyrolysis product (80° CT, 6' 3% SE-30) revealed 30-40 peaks with retention times from 0.3 min to 31.6 min. At 200° CT, peaks were observed with retention times of 2.13, 2.90, 3.3, 3.8, 4.2, 4.7, 6.0, 7.7, 10.9, 13.7, and 19.6 min. Using a 30' 3% SE-30 column, seven peaks that had retention times less than 11 min were observed at 75° CT.

Using a preparative aluminum column (20' × 3/8", 30% SE-30) three peaks were observed at 175° CT: t_R 1.90, 3.50, and 9.60 min. These compounds were collected in collection tubes equipped with steel rods and glass wool packing. The collection tubes were immersed in a bath of acetone—dry ice. From 300 mg of pyrolysis product injected, none of fraction 1 was collected; 11 mg of fraction 2 and 50 mg of fraction 3 were collected. The fractions were washed from the collection tubes with ether. An ir spectrum of fraction 2 (solution in CCl_4) exhibited absorptions at 2976, 2882, 1692, 1637, and 1587 k, among others; an ir spectrum of fraction 3 exhibited similar absorptions, except that an absorption at 1637 k was relatively more intense.

An nmr spectrum of fraction 2 was obtained. Absorptions occurred at τ 8.85 (d, J = 7 Hz), 8.1 (m), 7.9 (s), 7.3 (q), 6.55 (q), 3.9 (m), and 2.6 (m).

A pyrolysis of X-5108 (H^+), hexane-washed (1.1 g), was carried out under the same reaction conditions as described previously for the sodium salt; however, the reaction was done under reflux conditions.

The pyrolysate was extracted from the reaction vessel by trituration using chloroform and methanol (1:1). The solvents were evaporated, and the yield of the residue was 150 mg. This residue was chromatographed over a column of silicic acid (2 × 76 cm), eluting with chloroform. The fractions were collected manually, as the bands were fluorescent under 356 nm uv. The first fraction collected (22 mg of a yellow liquid with a smoky odor) had a blue-green fluorescence and gave one spot (R_F 0.78) in the tlc system chloroform—methanol (45:5). The first fraction had ir absorptions at 3448 and 2941 k, and several in the region 1724-1639 k, among others.

The second fraction collected (7 mg, purple-white fluorescence) exhibited R_F values of 0.58 and 0.37 in chloroform—methanol (45:5). This fraction was purified by preparative tlc, yielding 1.4 mg of an oil with an antiseptic smell. Absorptions in the ir spectrum were observed at 3448, 2941, 1715, and 1615 k, among others.

Permanganate Oxidation of X-5108

A sample of 1.00 g of X-5108 (H^+) that had been washed by precipitation using hexane was dispersed in 25 ml of water. A solution of 10% sodium bicarbonate (*ca.* 50 ml) was added to dissolve the X-5108. Aqueous potassium permanganate solution (2%) was added until the solution remained purple after being stirred at room temperature for 30 min; 225 ml of the permanganate solution was required. The solution was decolorized with oxalic acid, filtered using a Celite mat, and acidified with one milliliter of concentrated sulfuric acid to *p*H 3. The acidic solution was extracted with two 200-ml portions of ether. The ether

solution was dried; the chloroform was removed to yield 54 mg of a semi-crystalline *p*-phenylphenacyl product. Gas chromatography of this product (5' SE-30, CT 225°) resulted in retention times of 2.9, 5.9, 7.6 (relative area 5.6) and 9.4 (relative area 1); retention times of *p*-phenylphenacyl acetate, propionate, isobutyrate, and butyrate were 7.2, 9.4, 10.5, and 12.2 min; the products from a blank reaction had retention times of 2.8 and 5.2, a second injection of the distillation product resulted in retention times of 2.8, 5.8, 7.4 (relative area 5.1), and 9.3 (relative area 1).

The residue remaining in the distillation flask was dissolved in 40 ml of water. The solution was then continuously extracted with chloroform for 48 hr. The chloroform was evaporated to yield 13 mg of a foul-smelling, yellow gum interspersed with a white solid. The material was dissolved in 5 ml of methanol. To this was added 5 ml of redistilled 2,2-dimethoxypropane and two drops of concentrated hydrochloric acid. The solution was allowed to stand at room temperature overnight. The solvents were then evaporated, and the residue was dissolved in 20 ml of ether. The ethereal solution was washed with an equal volume of 5% aqueous sodium bicarbonate, then an equal volume of water. Ether (*ca.* 10 ml) was added to the organic layer, which was then dried. Evaporation of the ether layer yielded 8 mg of a yellow gum. Gas chromatography (12' OV-17) was carried out: a series (*ca.* 30) of peaks that were not well resolved at any of the conditions attempted was observed. A GC-MS experiment was attempted on a few of the more resolved peaks; the mass spectrum of the most intense peak (RT 4.3 at 190° CT) is given as Plate 3.

Catalytic Hydrogenation of X-5108

Hydrogenation Using Acetic Acid Solvent[13]

In a typical experiment, 5.0 g of 5% platinum on carbon catalyst was equilibrated in 25 ml of 75% acetic acid at 21° and 740 mm Hg. To this mixture 10.00 g of X-5108 (Na^+), as received, in 50 ml of 75% acetic acid, was added. The flask was rinsed with 25 ml of 75% acetic acid and this solution was added to the reaction vessel. The X-5108 was hydrogenated, with stirring, at atmospheric pressure. Within five minutes *ca.* 400 ml of hydrogen had been consumed. The rate of uptake began to decrease at *ca.* 1000 ml. After 1400 ml had been consumed, the rate became very slow. The mixture was then left to react overnight. Five similar reactions were carried out. The results are given in Table 4. Isolation of the hydrogenation products are described in a later section.

Table 4. Hydrogenations of X-5108 (Na^+) in 75% Acetic Acid (Corrected to STP)

Weight of X-5108	Moles of X-5108*	Liters H_2 Taken Up	Moles H_2 Taken Up	Moles H_2 / Moles H-5108
10.00 g	.0129	1.601	.0714	5.53
10.00 g	.0129	1.560	.0696	5.40
10.00 g	.0129	1.618	.0722	5.60
10.00 g	.0129	1.678	.0749	5.81
10.00 g	.0129	1.630	.0727	5.64
10.00 g	.0129	1.589	.0709	5.50
				5.58 Average

*Assuming 773 as the molecular weight.

evaporated, and, after a series of solvent extractions, chromatography procedures, and recrystallizations from various solvent systems, white crystals with mp 65-69.5° were obtained.

A mass spectrum of this material was obtained by the author; the mass spectrum (70 eV) exhibited *m/e* (rel intensity) 285 (17), 284 (82), 256 (26), 241 (21), 213 (17), 185 (10), 171 (8), 129 (41), 97 (25), 73 (100), and 60 (86). The fragmentation pattern was very similar to that of myristic acid;[15] the mass spectrum of stearic acid (lit[16] mp 69.4, mol wt 284) and palmitic acid (lit[17] mp 64, mol wt 256) would be expected to be very similar.

Hydrogenation Using 95% Ethanol Solvent[14]

In a hydrogenation flask, 1.001 g of 5% platinum on carbon was dispersed in 6 ml of 95% ethanol and allowed to equilibrate with hydrogen at 30° and 740 mm Hg. A solution of 2.002 g of X-5108 (Na^+), as received, in 25 ml of 95% ethanol was added to the flask and was washed in with *ca.* 8 ml of ethanol. (A plot of the hydrogen uptake as a function of time is given in Figure 2.) The mixture was filtered, and the catalyst was washed with chloroform. The filtrate and washings were subjected to ion-exchange chromatography and various solvent extractions. No crystalline or homogeneous material was isolated.

Hydrogenation Using Water Solvent[14]

The hydrogenation procedure was very similar to the preceding procedure: a solution of 2.004 g of X-5108 (Na^+), as received, in 25 ml of water and 1.012 g of 5% platinum on carbon dispersed in 10 ml of water was used. The hydrogenation was carried out at 30° and 740 mm Hg.

In another experiment,[14] 24.612 g of X-5108 (Na^+), as received, was hydrogenated using 13.160 g of 5% platinum on carbon at 19.5° and 740 mm Hg. The hydrogen uptake as a function of time is plotted in Figure 1.

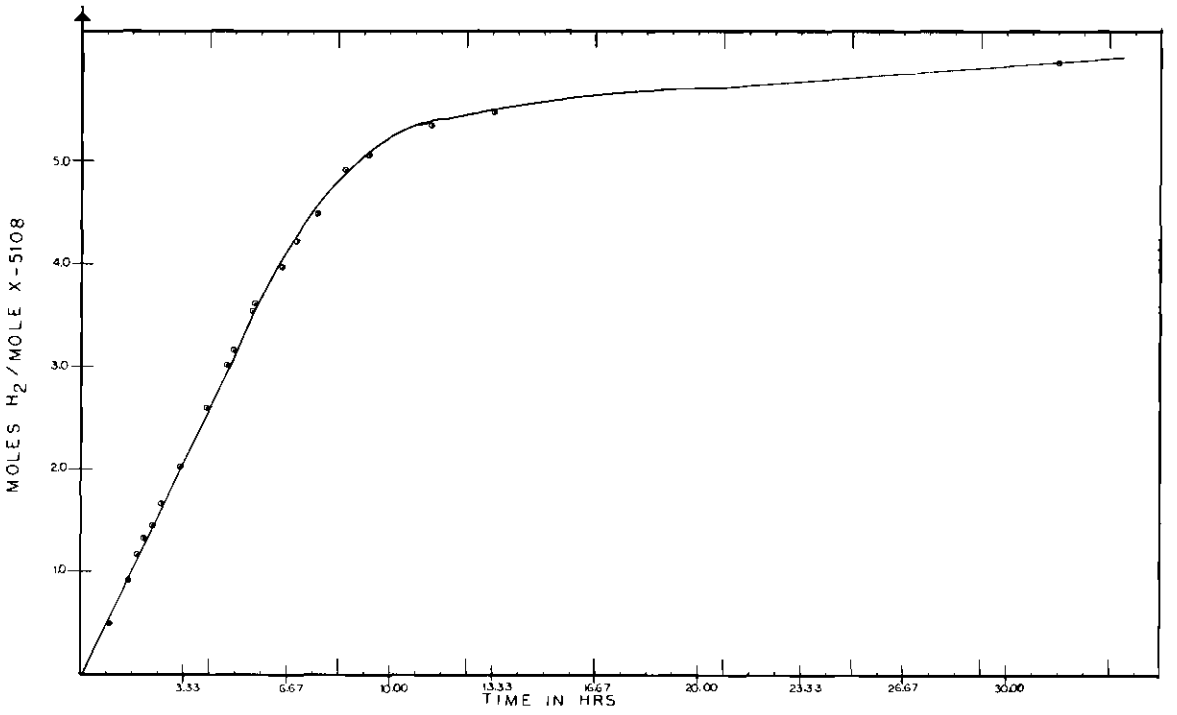

Figure 1. Hydrogenation of Antibiotic X-5108: 75% Aqueous Acid Solvent, 5% Pt/C Catalyst

In another experiment,[14] 40 g of X-5108 (Na^+), as received, was hydrogenated at atmospheric pressure using 20 g of platinum on carbon catalyst, with 75% acetic acid as solvent.

As part of the product-isolation procedure, the catalyst remaining in the sintered-glass funnel after filtration of the reaction mixture was washed with chloroform. The chloroform washings were

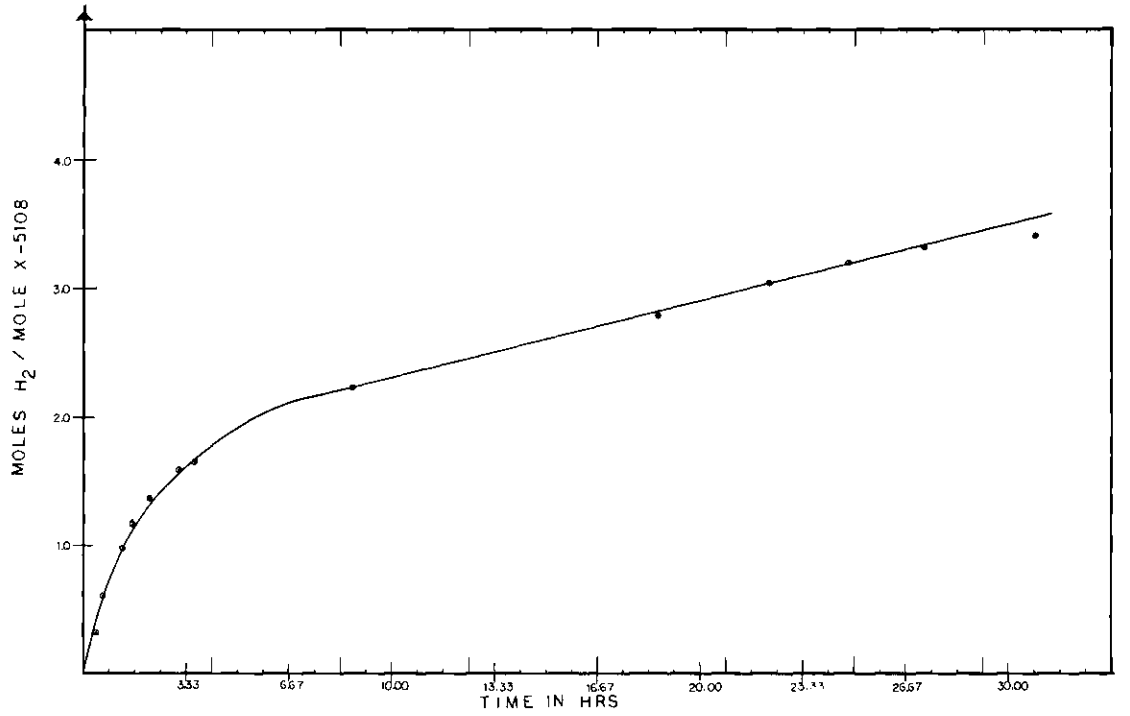

Figure 2. Hydrogenation of Antibiotic X-5108: 95% Ethanol Solvent, 5% Pt/C Catalyst

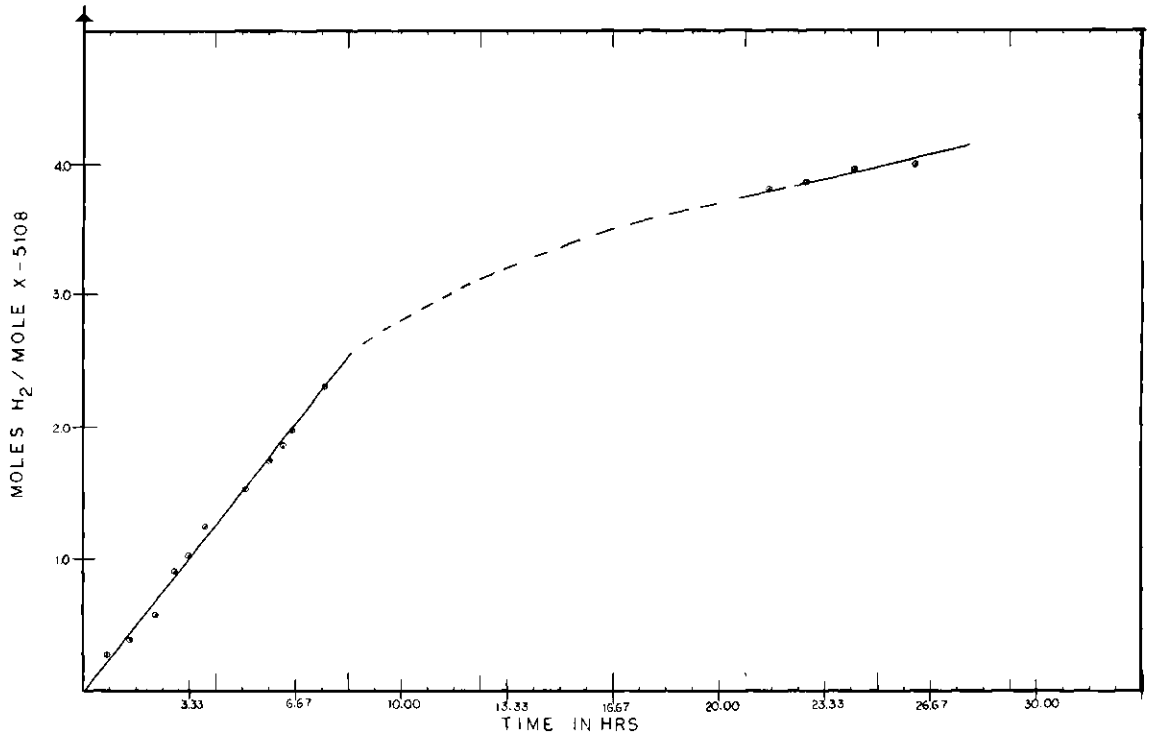

Figure 3. Hydrogenation of Antibiotic X-5108: Water Solvent, 5% Pt/C Catalyst

The results are given in Figure 3. The product of the reaction was treated in the manner described for the product of the preceding reaction.

Hydrogenation Using a Methanol-Water Solvent[18]

A sample of 0.556 g of X-5108 (Na^+), as received, was dissolved in 30 ml of methanol; to this solution was added 0.287 g of platinum on carbon in 50 ml of water. This mixture was hydrogenated at 40 psig for *ca.* 72 hr. A clear, pale yellow filtrate was obtained, which yielded 0.156 g of product after the solvents were evaporated. The black, tarry material remaining in the hydrogenation bottle was dissolved in chloroform and filtered *in vacuo* through a Celite mat. The mat was then washed with three 5-ml portions of chloroform. Evaporation of the chloroform yielded a residue (0.350 g), which was then chromatographed over a column of silicic acid (45 × 3 cm). The column was eluted with chloroform, then with increasing percentages of methanol in chloroform; 30-ml fractions were collected. After the solvents were evaporated from the collection flasks, 1 ml of methanol was added to each flask in order to combine the fractions. In all fractions, white flakes appeared; these flakes were collected by filtration, yielding 18 mg. An ir spectrum of the material (carbon tetrachloride solution) exhibited absorptions at 2950, 2865, and 1464 k. An nmr spectrum of the material exhibited absorptions at τ 8.78 and 9.1 (relative area 12:1, planimeter). Gas chromatography of the waxy material (6' SE-30, CT 245°) resulted in peaks with t_R of 7.6, 12.4, 16.4, 21.4, 28.1, 36.8, 48.0, 62.6, 93.9, and 105.6. The logarithms of these t_R produced a straight line when graphed against integral numbers.

Standard *n*-alkanes were used for comparison. These had the following t_R using the above conditions: eicosane, 1.9; docosane, 3.2; tetracosane, 4.5; octacosane, 17.9; triacontane, 28.95; and dotriacontane, 46.7.

The 156-mg residue left after evaporation of the pale yellow solution from the hydrogenation was then chromatographed over a column (30 × 3 cm) of silicic acid. Elution was carried out as before. A weight profile of the samples after evaporation of the solvents indicated that one major fraction (70.7% of the total weight) and several minor fractions had been collected. Thin-layer chromatography of the major fraction (5% methanol in chloroform eluent) demonstrated R_F values of 0.14, 0.35, and 0.89.

A repeat of the preceding experiment was carried out by the author using 0.502 g X-5108 (Na^+), as received, and 0.354 platinum on carbon catalyst. While flakes were isolated from the black, tarry substance as before, and the sample was submitted for MS. The mass spectrum exhibited clusters of ions spaced 14 mass units apart (at *m/e* 43, 57, 71, 85, 99 . . .) up to *m/e* 621.

A repeat of the experiment was carried out using 0.444 g of highly purified X-5108 (H^+) and 0.424 g of platinum on carbon. After the substance isolated from the black material was chromatographed, no white flakes were obtained.

Sequential Reductions of X-5108 (H^+)

A sample of 1.530 g of hexane-washed X-5108 (H^+) was dissolved in 60 ml of methanol. It was hydrogenated at 40 psig using 0.61 g of platinum on carbon dispersed in 50 ml of water. The mixture was hydrogenated for four days; it was then filtered through a Celite mat. The mat was washed with chloroform and the washings were added to the filtrate. The solvents were then evaporated to yield 1.544 g of a tan solid. This material was dissolved in 60 ml of tetrahydrofuran and slowly added to 1.5 g of lithium aluminum hydride in 50 ml of ethyl ether. The reaction was boiled under reflux overnight, with stirring.

After the mixture had been allowed to cool, the reaction flask was fitted with a straight condenser and water was added dropwise to the mixture, with stirring. When the lithium aluminum hydride had been decomposed, the product was filtered through a coarse sintered-glass funnel. The filter cake was washed with absolute ethanol and tetrahydrofuran. The combined washings and filtrate were evaporated to yield a brown oil (1.474 g).

The oil was triturated with *n*-hexane. Evaporation of the hexane yielded 36 mg of material, which was then used for gas chromatography (6 ft 3% OV-17, CT 165°). The sample yielded a broad aggregation of ill defined peaks centered at t_R 2.2, and a peak at t_R 7.8.

The material that was insoluble in hexane was also injected into the same column at the same temperature. Retention times occurred at 0.83, 2.5, 6.6, and 17.25. When the logarithm of these t_R were plotted *vs.* integral numbers, a straight line resulted. For comparison, a

series of *n*-paraffins (C_{12}, C_{14}, C_{16}, C_{18}) were injected at the same conditions, resulting in t_R of 0.9, 2.2, 6.3, and 16.6.

In a second experiment, 2.01 g of hexane-washed X-5108 (H^+) was dissolved in 50 ml of methanol; 70 mg of platinum on carbon in 50 ml of water was added. The mixture was hydrogenated at 40 psig for four days. The hydrogenation product was washed from the flask through a Celite mat on a coarse sintered-glass funnel with a mixture of chloroform and methanol. After evaporation of the solvents, the weight of the brown gum was 2.155 g.

The gum was dissolved in *ca.* 60 ml of tetrahydrofuran, and to this solution 2 g of lithium aluminum hydride in 60 ml of ether was added. The reaction mixture was stirred and boiled under reflux overnight. The excess lithium aluminum hydride was then decomposed as previously described.

The product was filtered through a coarse sintered-glass funnel and the filter cake was washed with ether. The resulting clear filtrate was evaporated to yield 0.77 g of a pale yellow gum. The filter cake was then dissolved in 10% sulfuric acid and extracted with 1-butanol. The extract was evaporated, yielding a brown gum weighing 1.60 g.

The brown gum was mixed with chloroform-methanol, and the mixture was filtered; then the filtrate was evaporated several times with cyclohexane. The residue was washed with three 50-ml portions of *n*-hexane. The hexane was evaporated to yield 13.6 mg of a colorless oil.

Gas chromatography of this material at 125° (12 ft 3.6% OV-17) demonstrated only small peaks on the flank of the large solvent peak. At 190°, a peak was observed at t_R 8.4.

The hexane-insoluble residue demonstrated GC peaks (5 ft 3% SE-30, CT 220°) at t_R of 0.8, 1.0, 2.75, 3.8, 5.6, 6.7, and 8.75 (not well separated). A 12 ft × 1/8 in. 3% OV-17 column was used for GC-MS. A technique of stepwise temperature programming was employed. The material was injected at a CT of 115°, and peak 1 was eluted. The CT was then slowly raised to 195°, and peak 2 was eluted. When the CT was raised to 215°, peak 3 was eluted; peaks 4, 5, and 6 were eluted after the CT had been raised to 250°. The GC-MS data are given in Table 5.

The pale yellow material from the filtrate previously described was injected into a 6 ft × 1/4 in. column of Porapak Q, 100/120 mesh (CT 200°). A peak was observed at a t_R of 4.7. The standards isobutyl alcohol, *t*-amyl alcohol, and *sec*-butyl alcohol were observed to have t_R of 5.3, 8.4, and 4.8, respectively, under the same conditions.

A sample of the pale yellow material (in methanol solution) was mixed with a drop of isobutyl alcohol and injected. Two peaks were observed at t_R of 4.9 and 5.5. A mixture of the yellow material and *sec*-butyl alcohol exhibited one peak, at t_R 4.6.

Preparation and Properties of Compound X

In a typical preparation of Compound X, 10 g of X-5108 (Na^+), as received, was dissolved in 100 ml of 75% acetic acid. To this solution 5 g of 5% platinum on carbon dispersed in 40 ml of water was added, and the residue was washed into the pressure bottle with 25 ml of 75% acetic acid. The X-5108 solution was hydrogenated for four days at a pressure of 10 psig. Generally, the pressure dropped to 5 psig after the first 30 min of hydrogenation.

Table 5. Mass Spectral Data from Sequential Reductions of X-5108

Components in Order of Elution	Relative Abundance of Peaks at *m/e* =						
	39	40	41	42	43	44	45
2	4	3	10	2	7	4	2
3	15	15	45	13	27	15	-
4	5	5	8	-	8	4	-
5	9	8	13	4	10	8	-
6	5	3	18	4	17	4	5
	53	54	55	56	57	58	59
2	3	2	9	46	10	2	3
3	14	9	30	63	22	9	-
4	5	-	7	7	-	-	-
5	6	-	18	10	10	-	10
6	5	2	22	6	11	-	12
	67	68	69	70	71	72	73
2	3	2	5	4	5	2	-
3	19	13	100	34	21	15	8
4	4	3	16	3	4	-	-
5	8	5	18	12	12	18	-
6	5	3	28	16	15	34	5
	83	84	85	86			
2	10	3	10	2	-		
3	30	16	69	9	-		
4	13	3	3	-	-		
5	13	5	9	-	-		
6	10	4	12	2	4		
	95	96	97	98	99	100	101
2	5	1	2	6	50	11	8
3	10	6	9	8	19	10	-
4	9	3	20	3	2	2	-
5	8	5	15	-	-	-	-
6	5	2	9	3	5	1	2

Table 5. Continued

	109	110	111	112	113	114	115
2	-	-	-	-	100	10	3
3	10	-	14	-	13	-	10
4	8	3	3	-	-	-	-
5	-	-	8	2	7	34	9
6	3	2	4	1	3	44	7

	121	122	123	124	125	126	127
2	-	-	3	5	2	1	2
3	9	9	10	-	-	45	10
4	8	4	7	8	5	4	-
5	-	8	-	6	6	8	6
6	-	-	-	3	5	3	5

	128	129	130	131	135	136	137
2	4	2	2	1	-	-	-
3	-	-	-	-	-	-	-
4	-	-	-	-	4	4	10
5	7	6	9	-	-	-	7
6	5	7	-	-	-	-	3

	138	139	140	141	142	143	144
2	-	-	-	-	-	-	-
3	-	-	-	-	-	12	-
4	4	9	6	4	-	-	-
5	3	5	3	3	100	20	6
6	2	2	1	1	100	18	4

	149	150	151	152	153	154	155
2	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
4	4	2	8	3	6	6	-
5	9	-	7	-	5	5	12
6	4	-	-	-	3	1	10

Table 5. Continued

	156	157	165	166	167	168	169
2	4	-	-	-	-	-	-
3	-	-	-	-	-	-	-
4	-	-	20	17	48	10	4
5	3	-	11	10	23	3	-
6	3	3	2	2	8	4	2

	177	178	179	180	181	182
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	4	13	7	7	6
5	4	-	5	3	3	-
6	-	-	-	-	-	-

	193	194	195	206	207	208	209
2	4	1	-	-	-	-	-
3	-	-	-	-	-	-	-
4	23	9	6	11	32	10	4
5	10	5	8	-	10	7	15
6	2	-	2	-	3	2	5

	210	211	212	213	214
2	-	-	-	-	-
3	-	28	9	-	-
4	-	-	-	-	-
5	4	4	-	-	9

Table 5. Continued

	221	222	223	224	225	226	227
2	-	-	-	-	-	-	-
3	-	-	-	-	9	6	-
4	39	11	5	-	-	-	-
5	12	-	-	-	6	7	14
6	2	-	-	-	2	-	4

	235	236	237	238	243	244	245
2	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
4	5	44	12	5	-	-	-
5	-	14	6	5	5	9	14
6	-	3	2	-	-	2	4

	249	250	251	264	296	315	392	393
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	100	20	5	28	-	-	-	-
5	25	8	-	14	6	9	8	5
6	5	2	-	4	-	2	2	-

The reaction mixture was filtered through a Celite mate in a sintered-glass funnel. The hydrogenation bottle was washed with 100 ml of 75% acetic acid, and this solution was used to wash the Celite mat. The filtrate was then filtered to remove insoluble particles, and the solvents were evaporated. Usually 11-12 g of a viscous brown liquid was obtained. This material was dissolved in a mixture of chloroform and methanol, and the solution was transferred to a smaller flask. The solvents were then evaporated. Cyclohexane was added to the residue and then evaporated; this procedure was repeated twice. The crude crystalline product could then be observed interspersed with a brown solid material. This product was then dried *in vacuo* over sodium hydroxide. In some cases, the material was dissolved in chloroform, and the solution was washed with 5% aqueous sodium bicarbonate. However, emulsions tended to form.

In some cases, the material was then dissolved in chloroform or 1% methanol in chloroform and chromatographed directly over a silica acid or Unisil column.

A method was then developed to avoid large-scale chromatography. The brown residue from the hydrogenation was dissolved in 95% ethanol and slurried with successive 1-g amounts of Darco (occasionally with gentle heating) until the solution became yellow in color. The yellow solution resulting from a typical hydrogenation of 10 g of X-5108 (Na^+) was then concentrated and chromatographed over a column (33 × 2 cm) of silicic acid and Darco (50:1). The adsorbents were homogenized thoroughly before use. Chloroform or 1% methanol in chloroform was used as

the eluent. The solvents were evaporated from the 25-ml fractions collected, and 1 ml of methanol was added to each flask. After the methanol was allowed to evaporate overnight, the flasks containing crystalline material were combined. Generally, *ca.* 2 g of the impure crystalline compound X was obtained. The compound was purified by successive recrystallizations from methanol-water, using Darco during the first recrystallizations. The method of recrystallization was as follows: the material was dissolved in methanol, and water was added until the solution became cloudy. Vigorous shaking caused flocculent crystals to appear. The procedure was repeated if the crystal mass was too voluminous. The solution was then warmed on a hot plate (Darco was added if necessary) to the boiling point and filtered rapidly. The solution was allowed to cool slowly. If the solution was cooled rapidly, very tiny needles appeared. If no crystals appeared, a seed crystal was added, and growth was usually rapid. The crystals were needles; varying lengths and thicknesses were obtained from various conditions, ranging from a fine glass-wool appearance to thick clusters of flat needles.

The product purified in this way had a mp of 101-104° and had an R_F value of 0.50 (visible with iodine vapor only) in the system ether--*t*-butyl alcohol (45:1). If the crystalline product had a lower melting point, it was washed with very cold (0°) cyclohexane and recrystallized again from methanol-water. The compound was soluble in warm cyclohexane and could be recrystallized from this solvent; however, the crystals were extremely fine needles that tended to stick together. Benzene was also used as a recrystallizing solvent, but again the crystals obtained

were not tractable. The compound was insoluble in water, very soluble in chloroform, and soluble in ether when pure. It was soluble in hexane and petroleum ether, but not very soluble in cold acetone.

Analysis of compound X indicated the formula $C_{16}H_{28}O_5$ (calcd, C, 63.97; H, 9.40, O, 26.63; found, C, 63.76; H, 9.18; 0, 27.00). The saponification equivalent was found to be 432 (0.69 groups), active hydrogen analysis indicated 1.36% (4.08 active hydrogens), and *C*-methyl analysis gave a value of 7.84% (1.57 groups). Compound X was also optically active, having a rotation $[\alpha]_D^{22}$ - 17° (*c* 1.0, EtOH).

Spectral Studies of Compound X

Compound X had a uv absorption at λ_{max}^{EtOH} 217 nm, ϵ 1200. In the ir spectrum, (KBr pellet) absorptions were observed at 3559, 3413, 2941, 2882, 1751 and 1639 k, among others (Plate 4); in chloroform solution the absorptions occurred at 3650, 3484, 2976, and 1779 k, among others.

The mass spectrum of compound X exhibited a molecular ion at *m/e* 300; a high resolution spectrum demonstrated that the exact mass was 300.1931 (calcd for $C_{16}H_{28}O_5$, 300.1937). The low resolution mass spectrum is given as Plate 5; the high resolution data are given as Table 6.

The nmr spectrum of compound X (deuterochloroform solution) exhibited absorptions centered at τ 9.10, 8.70, and 8.22 (22 H total); 7.42 (1 H); 6.55, 6.35, and 6.10 (3 H total); and 5.60 and 5.33 (2 H total). This spectrum is given as Plate 6. At 100 MHz in pyridine-d_5, absorptions occurred at τ 9.15 (t, 3 H), 8.77 and 8.48 (17 H total); 7.90 (quint, 2 H, J = 7.5); 7.15 (t, 1 H, J = 7.0); 6.12 (br t, 1 H, J = 5.0); 5.89 (d, 1 H, J = 4.0); 5.10 (d, 1 H, J = 4.0); 4.75 (very

Table 6. High Resolution Mass Spectrum of Compound X

Nominal Mass	Ratio $\frac{300}{293} + \frac{282}{281}$	Measured	Calculated	Error
300	$\frac{300}{292.9825} = 1.024611$	300.1930923	300.193671 $C_{16}H_{28}O_5$	~0.6 mmu
282	$\frac{282}{280.9825} = 1.004268$	282.181733	282.183106 $C_{16}H_{26}O_4$	~1.4 mmu

Mass measured at 75 ppm resolution.

CALCULATED MASS	ERR	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
231.1703	-2.41	15/1	22	0	1	231.1680	6	+++++++++++++
231.1595	-3.72	12/0	23	0	4	231.1558	7	+++++++++++++
231.1551	.76	11/1	22	0	4			
223.1361	2.65	12/0	21	0	4	223.1388	8	+++++++++++++
213.1496	-1.09	12/0	21	0	3	213.1479	11	+++++++++++++
213.1445	3.38	11/1	20	0	3			
200.1048	.88	10/0	16	0	4	200.1057	17	+++++++++++++++
143.0976	-2.58	9/1	14	0	3	143.0950	11	+++++++++++++
142.0942	.79	10/0	14	0	3	142.0949	13	+++++++++++++++
174.0528	1.03	7/0	10	0	5	174.0538	9	+++++++++++++
173.1541	- .30	10/0	21	0	2	173.1538	9	+++++++++++++
172.1462	-1.15	10/0	20	0	2	172.1451	5	++++++++++++
172.1418	3.32	9/1	19	0	2			
172.1099	.79	9/0	16	0	3	172.1107	8	+++++++++++++
171.1021	.67	9/0	15	0	3	171.1027	6	+++++++++++++
168.1514	.09	11/0	20	0	1	168.1514	5	++++++++++++
167.1435	2.19	11/0	19	0	1	167.1457	11	+++++++++++++
167.0708	1.95	9/0	11	0	3	167.0727	7	+++++++++++++
158.0578	-2.47	7/0	10	0	4	158.0554	10	+++++++++++++
158.0534	2.01	6/1	9	0	4			
157.0500	-2.92	7/0	9	0	4	157.0471	7	+++++++++++++
157.0456	1.52	6/1	8	0	4			
156.1469	-1.40	9/1	19	0	1	156.1455	5	++++++++++++
155.1435	- .97	10/0	19	0	1	155.1425	6	+++++++++++++
155.1391	3.47	9/1	18	0	1			
155.1026	- .64	8/1	14	0	2	155.1020	13	+++++++++++++++
154.0993	-2.16	9/0	14	0	2	154.0971	17	+++++++++++++++
154.0948	2.28	8/1	13	0	2			

CALCULATED MASS	ERR	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
153.0551	- .21	8/0	9	0	3	153.0549	8	+++++++++++++
151.1441	- .12	14/1	18	0	0	151.1440	6	+++++++++++++
MISSING REFERENCE								
149.1329	-1.28	11/0	17	0	0	149.1317	8	+++++++++++++
149.1234	3.20	10/1	16	0	0			
137.1330	-1.15	11/0	17	0	0	137.1318	12	+++++++++++++++
137.1235	3.29	9/1	16	0	0			
135.1173	-1.03	10/0	15	0	0	135.1163	5	++++++++++++
135.1129	3.44	9/1	14	0	0			
133.0500	.97	5/0	9	0	4	133.0510	9	+++++++++++++
NO COMP CALC						130.0494	7	+++++++++++++
129.0506	- .24	5/1	8	0	3	129.0504	18	+++++++++++++++
125.0473	-2.16	6/0	8	0	3	123.0451	16	+++++++++++++++
128.0428	2.31	5/1	7	0	3			
127.1486	-2.91	9/0	19	2	0	127.1457	10	+++++++++++++
127.1441	1.57	8/1	18	0	0			
127.0394	-2.76	6/0	7	0	3	127.0367	10	+++++++++++++
127.0350	1.72	5/1	6	0	3			
126.1363	-1.70	8/1	17	0	0	126.1346	5	++++++++++++
126.1044	-3.61	8/0	14	0	1	126.1008	10	+++++++++++++
126.0999	.35	7/1	13	0	1			
125.0966	-3.35	8/0	13	0	1	125.0932	7	+++++++++++++
125.0921	1.11	7/1	12	0	1			
125.0602	-3.60	7/0	9	0	2	125.0566	6	+++++++++++++
125.0557	.86	6/1	8	0	2			
124.1251	-3.37	9/0	16	0	0	124.1218	11	+++++++++++++
124.1207	1.11	8/1	15	0	0			
123.1173	-2.01	9/0	15	0	0	123.1153	7	+++++++++++++
123.1128	2.47	8/1	14	0	0			
115.0758	1.26	6/0	11	0	2	115.0771	3	+++++++++++++

CALCULATED MASS	PPM	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
115.0394	1.38	5/0	7	0	3	115.0403	10	+++++++++++++
113.0966	1.49	7/0	13	0	1	113.0980	9	+++++++++++++
113.0602	3.65	6/0	9	0	2	113.0634	9	+++++++++++++
112.0887	.44	7/1	12	0	1	112.0892	6	+++++++++++++
112.0524	-1.87	6/0	8	0	2	112.0505	8	+++++++++++++
112.0479	2.59	5/1	7	0	2			
111.1173	.54	8/0	15	0	0	111.1179	5	++++++++++++
111.1137	- .32	8/1	14	0	5			
111.0809	- .76	7/0	11	0	1	111.0802	5	++++++++++++
111.0765	3.69	6/1	10	0	1			
111.0445	2.04	6/0	7	0	2	111.0466	13	+++++++++++++++
109.1031	2.85	8/1	16	0	5	109.1059	10	++++++++++++
109.0653	3.39	7/0	9	0	1	109.0692	7	++++++++++++
108.0938	3.64	8/0	12	0	0	108.0975	6	++++++++++++
108.0952	2.25	8/1	15	0	5			
107.0860	1.34	8/0	11	0	0	107.0874	9	++++++++++++
107.0874	- .04	8/1	14	0	5			
105.0187	2.10	3/0	5	0	4	105.0208	8	++++++++++++
102.0680	.28	5/0	10	0	2	102.0683	10	++++++++++++
101.0966	-1.40	6/0	13	0	1	101.0952	9	++++++++++++
101.0921	3.06	5/1	12	0	1			
101.0602	1.23	5/0	9	0	2	101.0615	9	++++++++++++
101.0557	-3.03	4/1	8	0	2	101.0527	5	++++++++++++
100.0523	-1.14	5/0	8	0	2	100.0512	17	+++++++++++++++
100.0479	3.32	4/1	7	0	2			
100.0160	.63	4/0	4	0	3	100.0167	10	++++++++++++
99.0809	- .03	6/0	11	0	1	99.0809	7	++++++++++++
99.0764	.15	5/1	10	0	1	99.0766	5	++++++++++++

CALCULATED MASS	PPM	C12/13	R	N	O	MEASURED MASS	NO. PTS	INTENSITY
99.0445	-1.61	5/0	7	0	2	99.0429	7	+++++++++++++
99.0401	2.35	4/1	6	0	2			
98.1095	.19	7/0	14	0	0	98.1097	8	+++++++++++++
98.0731	1.96	6/0	10	0	1	98.0742	7	+++++++++++++
98.0367	- .13	5/0	6	0	2	98.0366	9	+++++++++++++
97.1017	- .64	7/0	13	0	0	97.1010	13	+++++++++++++++
97.0972	3.84	6/1	12	0	0			
97.0653	-2.94	6/0	9	0	1	97.0623	8	+++++++++++++
97.0603	1.52	5/1	8	0	1			
97.0289	.10	5/0	5	0	2	97.0290	11	+++++++++++++
96.0574	-3.17	6/0	8	0	1	96.0543	5	++++++++++++
96.0530	1.29	5/1	7	0	1			
95.0860	- .43	7/0	11	0	0	95.0855	8	+++++++++++++
95.0816	3.93	6/1	10	0	0			
95.0816	- .53	6/1	10	0	0	95.0810	5	++++++++++++
95.0496	- .22	6/0	7	0	1	95.0494	5	++++++++++++
95.0496	-3.76	6/0	7	0	1	95.0458	5	++++++++++++
95.0452	.70	5/1	6	0	1			
93.0704	- .22	7/0	9	0	2	93.0701	11	+++++++++++++
91.0547	-1.65	7/0	7	0	0	91.0537	7	+++++++++++++
91.0502	3.43	6/1	6	0	0			
89.0238	-2.38	3/0	5	0	3	89.0214	5	++++++++++++
89.0193	2.10	2/1	4	0	3			
88.0479	- .45	3/1	7	0	2	88.0474	6	+++++++++++++
87.0445	-2.59	4/0	7	0	2	87.0419	12	+++++++++++++++
87.0401	1.87	3/1	6	0	2			
86.0731	- .03	5/0	10	0	1	86.0731	5	++++++++++++
86.0686	-1.05	4/1	9	0	1	86.0676	10	+++++++++++++
86.0367	-2.94	4/0	6	0	2	86.0338	7	+++++++++++++
86.0322	1.51	3/1	5	0	2			

CALCULATED MASS	ERR	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
85.1017	-3.44	6/0	13	0	0	85.0982	10	+++++++++++++
85.0972	1.03	5/1	12	0	0			
85.0653	-2.33	5/0	9	0	1	85.0629	18	+++++++++++++++
85.0608	2.13	4/1	8	0	1			
85.0239	-2.45	4/0	5	0	2	85.0264	9	+++++++++++++
85.0244	2.01	3/1	4	0	2			
84.0938	-3.13	6/0	12	0	0	84.0906	13	+++++++++++++++
84.0893	1.29	5/1	11	0	0			
84.0574	-2.19	5/0	8	0	1	84.0552	17	+++++++++++++++
84.0530	2.27	4/1	7	0	1			
84.0211	- .61	4/0	4	0	2	84.0205	5	+++++++++++++
84.0166	3.86	3/1	3	0	2			
83.0860	- .61	6/0	11	0	0	83.0854	19	+++++++++++++++
83.0815	3.86	5/1	10	0	0			
83.0496	-1.20	5/0	7	0	1	83.0484	15	+++++++++++++++
83.0451	3.26	4/1	6	0	1			
82.0782	.61	6/0	10	0	0	82.0788	5	+++++++++++++
82.0732	-3.66	6/0	10	0	0	82.0745	8	+++++++++++++
82.0737	.82	5/1	9	0	0			
81.0704	.74	6/0	9	0	0	81.0711	14	+++++++++++++
81.0340	- .09	5/0	5	0	1	81.0339	13	+++++++++++++++
79.0547	- .80	6/0	7	0	0	79.0539	13	+++++++++++++++
79.0502	3.67	5/1	6	0	0			
77.0391	-1.15	6/0	5	0	0	77.0379	9	+++++++++++++
77.0346	3.32	5/1	4	0	0			
76.0160	-1.38	2/0	4	0	3	76.0146	6	+++++++++++++
76.0115	3.09	1/1	3	0	3			
NO COMP CALC						74.0622	8	+++++++++++++
74.0156	-1.23	6/0	2	0	0	74.0144	5	+++++++++++++
74.0111	3.25	5/1	1	0	0			
73.0653	-1.60	4/0	9	0	1	73.0637	24	+++++++++++++++
73.0608	2.86	3/1	8	0	1			

CALCULATED MASS	PPM	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
73.0289	.71	3/0	5	0	2	73.0296	5	+++++++++++
73.0239	-1.96	3/0	5	0	2	73.0269	5	+++++++++++
73.0244	2.59	2/1	4	0	2			
72.0575	- .38	4/0	8	0	1	72.0571	20	++++++++++++++++
71.0860	- .36	5/0	11	0	0	71.0856	19	+++++++++++++++
71.0496	1.22	4/0	7	0	1	71.0509	17	+++++++++++++++
71.0132	-1.44	3/0	3	0	2	71.0118	5	+++++++++++
71.0083	3.59	2/1	2	0	2			
70.0782	.73	5/0	10	0	0	70.0789	16	+++++++++++++++
70.0737	- .51	4/1	9	0	0	70.0732	8	+++++++++++++
70.0418	.74	4/0	6	0	1	70.0425	14	+++++++++++++++
70.0418	-3.39	4/0	6	0	1	70.0379	5	+++++++++++
70.0373	.57	3/1	5	0	1			
69.0704	1.72	5/0	9	0	0	69.0721	18	+++++++++++++++
69.0659	- .03	4/1	8	0	0	69.0659	5	+++++++++++
69.0343	1.86	4/0	5	0	1	69.0353	12	+++++++++++++++
68.0625	2.59	5/0	8	0	0	68.0651	18	+++++++++++++++
68.0625	-2.65	5/0	8	0	0	68.0599	5	+++++++++++
68.0581	1.81	4/1	7	0	0			
67.0547	3.09	5/0	7	0	0	67.0573	16	+++++++++++++++
NO COMP CALC						66.0516	6	+++++++++++
NO COMP CALC						65.0441	13	+++++++++++++
NO COMP CALC						65.0258	6	+++++++++++
NO COMP CALC						60.0301	11	+++++++++++
NO COMP CALC						59.0576	8	+++++++++++
NO COMP CALC						59.0244	8	+++++++++++
NO COMP CALC						59.0214	6	+++++++++++

CALCULATED MASS	ERR	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
NO COMP CALC						58.0352	5	++++++++++++
NO COMP CALC						58.0324	5	++++++++++++
NO COMP CALC						58.0526	5	++++++++++++
NO COMP CALC						58.0486	18	+++++++++++++++
NO COMP CALC						58.0156	15	+++++++++++++++
NO COMP CALC						57.0812	16	+++++++++++++++
NO COMP CALC						57.0758	7	++++++++++++
NO COMP CALC						57.0449	16	+++++++++++++++
NO COMP CALC						56.0737	17	+++++++++++++++
NO COMP CALC						56.0675	5	++++++++++++
NO COMP CALC						56.0383	7	++++++++++++
NO COMP CALC						56.0330	6	++++++++++++
NO COMP CALC						55.0661	19	++++++++++++++++
NO COMP CALC						55.0294	21	+++++++++++++++
54.0635	-3.99	0/1	9	0	2	54.0596	7	++++++++++++
53.0557	-2.31	0/1	8	0	2	53.0534	13	+++++++++++++++
52.0479	-1.93	0/1	7	0	2	52.0460	7	++++++++++++
51.0401	- .29	0/1	6	0	2	51.0399	13	+++++++++++++++
51.0234	-2.19	4/0	3	0	0	51.0213	17	+++++++++++++++
51.0190	2.36	3/1	2	0	0			
50.0322	2.85	0/1	5	0	2	50.0351	7	++++++++++++
50.0156	-1.85	4/0	2	0	0	50.0137	5	++++++++++++
50.0111	2.62	3/1	1	0	0			
MISSING REFERENCE								
NO COMP CALC						45.0573	12	+++++++++++++++
NO COMP CALC						45.0212	13	+++++++++++++++

CALCULATED MASS	ERR	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
44.0037	- .83	0/0	12	0	2	44.0028	12	+++++++++++++
NO COMP CALC						44.0517	10	+++++++++++++
NO COMP CALC						44.0471	13	+++++++++++++
NO COMP CALC						44.0155	7	+++++++++++++
NO COMP CALC						44.0129	9	+++++++++++++
NO COMP CALC						43.0405	20	+++++++++++++++
NO COMP CALC						43.0438	22	+++++++++++++++
NO COMP CALC						42.0748	12	+++++++++++++
42.0659	2.84	0/0	10	0	2	42.0709	8	+++++++++++++
NO COMP CALC						42.0368	5	+++++++++++
NO COMP CALC						41.0670	25	+++++++++++++++
NO COMP CALC						40.0621	6	+++++++++++++
NO COMP CALC						40.0571	8	+++++++++++++
NO COMP CALC						39.0534	22	+++++++++++++++

LIMIT OF DATA

br, 1 H); and 1.55 (br abs, 1 H). The spectrum was determined within 4 hr of sample preparation. The multiplicities and coupling constants were obtained by expansions. The spectrum of compound X pyridine-d_5 is given as Plate 6a.

After standing for six days, the spectrum of the pyridine-d_5 solution of X was again determined. The absorption at τ 1.5 had disappeared; the absorption at τ 4.75 had increased to 2 H. When the sample was concentrated, the absorption at τ 4.75 shifted downfield.

A quantity of 0.1 ml of deuterium oxide was then added to the solution, and the spectrum was determined after 1 hr. The only change was the disappearance of the absorption at τ 4.75 and the appearance of the HOD peak. The spectrum was again determined after three days, and after this time the triplet at τ 7.15 had disappeared.* The doublets at τ 5.89 and 5.10 had the appearance of triplets; expansion demonstrated triplets with J = 4.0. However, when the spectrum was determined using the 60 MHz instrument, four-line absorptions were observed, rather than triplets. The absorption centered at τ 5.08 had J = 2.6 and J = 4.0; the absorption centered at τ 5.79 had J = 1.8 and J = 4.0.

An experiment was then carried out in which the nmr spectra of ethyl acetoacetate and 4-valerolactone were (1) determined in pyridine-d_5, (2) determined one hour after 0.1 ml of deuterium oxide had been added, and (3) determined four days after 0.1 ml of deuterium oxide had

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*The experiment was repeated to determine the approximate rate of disappearance of the triplet. It was found that disappearance took place after one day.

been added. The results are given in Table 7.

Spectra of samples of ethyl lactate and triethyl citrate (α- and β-hydroxy esters) were also determined using pyridine solvent. The spectra were also determined after addition of deuterium oxide as previously described. It was found that the integration did not change ten days after the addition of deuterium oxide.

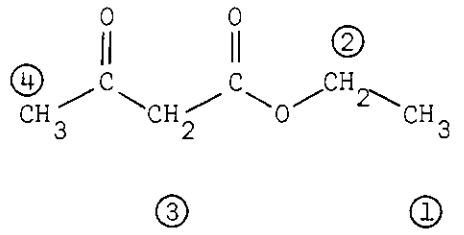
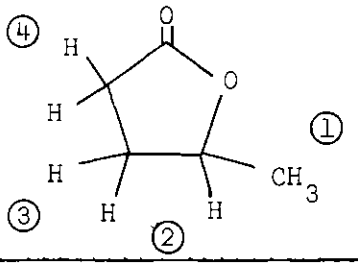
Spin-decoupling experiments using compound X in pyridine-d_5 were also carried out. When the absorption at τ 5.83 was irradiated, the doublet at τ 5.12 collapsed to a singlet. No other change was apparent in the spectrum. When the absorption at τ 5.12 was irradiated, the doublet at τ 5.83 collapsed to a singlet. Irradiation of other absorptions was also attempted, but the close proximity of the absorptions made this experiment unfeasible.

Spin decoupling was also accomplished using the solution of compound X in pyridine-d_5 after equilibration with deuterium oxide. When the quintet at τ 7.90 was irradiated, no change was observed downfield. When the triplet at τ 5.83 was irradiated, the absorption at τ 5.12 collapsed to a doublet. When the triplet at τ 5.12 was irradiated, the absorption at τ 5.83 collapsed to a doublet.

The region 60-140 Hz was then expanded, using pyridine-d_5 as the solvent. A distorted triplet was observed centered at τ 9.17, while adsorptions at τ 8.96, 8.89, 8.83, 8.78, 8.76, and 8.69, of different intensities, were observed.

The solvents were evaporated from the solution used for nmr *in vacuo* at room temperature. An ir spectrum of the resultant yellow gum

Table 7. Integral Areas of Protons of Ethyl Acetoacetate and 4-Valerolactone Before and After Equilibration with Deuterium Oxide*†

				
	Area, Before Addition of D_2O	Area, 4 Days After Addition of D_2O	Area, Before Addition of D_2O	Area, 4 Days After Addition of D_2O
1	3.0	3.0	3.0	3.0
2	2.5	1.6	1.4	1.1
3	2.3	0.4	2.6	2.6
4	3.0	2.5	2.7	2.2

*All areas were calculated assuming absorption 1 = 3 protons.

†The closeness of the peaks in the 4-valerolactone spectrum made correct determination of the integration difficult.

was obtained; a strong, broad absorption centered at 2400 k was observed. This was the only discernible change from the ir spectrum of compound X. A uv spectrum was attempted; however, residual pyridine (λ_{max}^{EtOH} 257, log ϵ 3.43)[19] and strong end absorption obscured any absorption resulting from deuterated compound X.

A mass spectrum was obtained of the residue; the mass spectrum is given as Plate 7.

Chemical Tests

Compound X gave a positive Fehling's test, a positive hydroxamic acid test, a negative Benedict's test, and a negative ferric chloride test. A solution of compound X in methanol slowly decolorized bromine water within 1 hr, although no immediate reaction was observed. A blank produced no decoloration.

A periodic acid test was performed, and positive results were obtained after standing 15 min. The test was then repeated, using 22 mg of compound X, 10 ml of periodic acid reagent,[20] and 5 ml of nitric acid. The filtrate was treated with freshly prepared 2,4-dinitrophenylhydrazine reagent, and the orange precipitate was filtered. The filtrate was again treated with the reagent, and the precipitate was filtered.

The combined precipitates were washed with water and dried *in vacuo*, yielding 48 mg of an orange-red powder. Using chloroform as eluent, tlc of this material indicated R_F values of 0.0, 0.1, and 0.28.

The products were then chromatographed over a column of silicic acid (32 × 1.8 cm); four distinct bands were collected. Fractions 1-2 (Band 1) were combined to yield 2 mg of material with an R_F value of 0.42 (chloroform); fractions 3-4 (Band 2), 11 mg total, had an R_F value of 0.30 and a mp of 128-138°, fractions 10-13 (Band 3), 9 mg total, had an R_F value of 0.13 and a mp of 206-212°; fractions 14-18 (Band 4), 14 mg total, had an R_F value of 0.0 and a mp of 176-179°.

Mass spectra were obtained of Bands 2, 3, and 4. Band 2 had *m/e* (rel intensity) 238 (25), 224 (100), 181 (22), 152 (20), 131 (30), 122 (54), 104 (48), 91 (51), 79 (91), and 78 (92), among others; Band 3

had *m/e* (rel intensity) 281 (6), 267 (2), 256 (3), 240 (2), 226 (3), 207 (15), 198 (10), 183 (16), 164 (18), 154 (25), 153 (28), 152 (27), 137 (29), 127 (21), 107 (35), 191 (60), 79 (100), and 75 (95), among others; Band 4 had *m/e* (rel intensity) 183 (100), 167 (10), 153 (64), 149 (23), 137 (15), 107 (40), 91 (66), and 79 (41), among others.

A mixture of *ca.* 2:1 of an authentic sample of acetone 2,4 dinitrophenylhydrazone and an authentic sample of acetaldehyde 2,4 dinitrophenylhydrazone had a mass spectrum which exhibited ions at *m/e* (rel intensity) 238 (47), 224 (100), 208 (3), 181 (15), 152 (14), 131 (5), 122 (20), 104 (11), 91 (15), 79 (100), and 78 (86), among others.

The mass spectrum of a sample of 2,4 dinitroaniline (lit[21] mp 176°, mw 183) was essentially like that of Band 4.

Attempted Preparation of the Trimethylsilyl Derivative of Compound X

The method of Sweeley *et al.*[7] was employed in an attempt to prepare the trimethylsilyl ether of compound X: 10 mg of compound X, dissolved in 1 ml of dry pyridine, was treated with 0.2 ml of HMDS and 0.1 ml of trimethylchlorosilane. After standing for 4 days, the solution had t_R of 3.9, 4.7, 5.9, and 9.8 (CT 250°, 5 ft 3% SE-30).

It was possible to obtain gc-mass spectra of the third and fourth peaks. The results are given in Table 8.

Basic Hydrolysis of Compound X

A sample of 50 mg of compound X was treated with 2 ml of 20% sodium hydroxide, and the mixture was heated at *ca.* 70° for 6 hr. After

Table 8. Mass Spectra of Products from Attempted Trimethylsilylation of Compound X

Relative Abundance at *m/e* =	Components in Order of Elution		Relative Abundance at *m/e* =	Components in Order of Elution		Relative Abundance at *m/e* =	Components in Order of Elution	
	3	4		3	4		3	4
39	14	30	85	6	48	140	-	15
40	4	7	86	-	5	141	-	3
41	25	100	87	-	-	143	6	-
42	8	14	95	11	16	153	-	3
43	20	92	96	5	4	154	-	3
44	5	10	97	14	79	155	7	53
45	-	7	98	6	10	156	9	9
53	10	28	99	5	13	157	54	23
54	5	7	100	-	3	158	5	-
55	20	63	109	-	5	165	9	-
56	12	24	110	-	-	167	12	9
57	9	40	111	19	5	172	10	-
58	-	8	112	-	3	193	-	3
65	-	5	113	12	5	199	100	-
66	-	2	117	5	-	200	24	-
67	10	16	123	7	-	201	9	-
68	5	7	124	-	12	207	-	5
69	35	86	125	5	17	221	-	5
70	9	19	126	5	5	236	6	5
71	8	44	127	6	19	249	8	9
72	-	9	128	-	56	253	6	9
73	18	-	129	6	5	264	-	5
75	10	-	137	7	13	282	-	9
83	14	29	138	-	2	283	-	1
84	7	7	139	5	5			

cooling, the mixture was diluted with 10 ml of water and extracted with three 10-ml portions of ether.

The aqueous layer was then made acidic (*p*H 1) with dilute sulfuric acid and extracted with three equal volumes of diethyl ether. The ether extract was dried and the solvent was evaporated.

The residue was dissolved in 5 ml of methanol, and to this solution 5 ml of 2,2-dimethoxypropane and two drops of concentrated hydrochloric acid were added. The reaction mixture, after standing overnight, was then injected directly into the gas chromatograph (5 ft × 1/8 in. 3% SE-30, CT 165°). Peaks were observed with t_R of 3.2, 4.0, 4.8, 5.3 sh, 5.8, 7.1, 9.4, and 12.9 (large asymmetric peak). For GC-MS, a programming rate of 2°/min was employed, beginning at 170°. At 200° the temperature was maintained. Nine peaks were observed. Eight mass spectra were obtained; the results are given in Table 9.

Attempted Preparation of Bromoacetyl X

A solution of 100 mg (0.33 mmoles) of thoroughly dry compound X in 1 ml of dry pyridine was prepared, and to this solution was added 0.10 ml (1.15 mmoles) of bromoacetyl bromide from a freshly-opened bottle. Reaction took place immediately, and the solution became black and tarry.

The reaction residue was then dissolved in 15 ml of chloroform and washed with two 10-ml portions of 5% sodium bicarbonate, two 10-ml portions of 1 *N* hydrochloric acid, and 10 ml of distilled water. The organic layer was then treated with small portions of Darco, Celite, and magnesium sulfate; after filtration, the solvents were evaporated to yield 61 mg of a brown oil.

Table 9. Mass Spectra of the Products from a Basic Hydrolysis of Compound X

Components in Order of Elution	Relative Intensity at *m/e* =													
	39	40	41	42	43	44	45	51	52	53	54	55	56	57
1	18	10	53	14	25	11	-	-	-	11	5	54	20	16
2	6	4	29	6	28	6	5	-	-	4	2	27	37	20
3	11	7	33	7	40	10	5	-	-	9	4	38	18	12
4	10	5	39	6	21	5	3	-	-	10	4	32	11	10
5	19	7	47	7	29	7	-	4	3	18	6	48	9	11
6	7	3	26	8	17	5	3	-	-	4	2	23	7	25
7	3	1	13	3	12	3	1	-	-	3	1	15	6	8
9	9	2	38	5	47	5	3	-	-	9	3	34	10	32

	58	59	60	65	67	68	69	70	71	72	73	74	75	77
1	5	-	-	-	10	30	100	37	23	5	-	-	-	-
2	4	71	8	-	4	6	66	22	12	26	4	1	-	-
3	4	8	-	-	11	18	66	29	61	9	6	6	-	-
4	3	12	-	-	10	6	88	27	10	3	-	10	3	4
5	-	6	-	6	22	8	96	14	10	-	4	-	-	-
6	3	-	-	3	8	5	69	16	9	3	18	5	8	6
7	1	8	-	-	4	3	20	14	9	26	3	-	-	-
9	4	2	-	2	8	4	73	19	48	8	3	-	-	3

Table 9. Continued

	83	84	85	86	87	88	95	96	97	98	99	100	101	102
1	27	10	6	-	-	-	11	5	13	7	-	-	-	-
2	14	10	7	1	5	-	5	2	6	6	5	4	4	4
3	33	10	14	-	-	-	13	6	18	9	9	-	7	3
4	38	9	7	3	4	-	15	5	100	11	8	-	-	-
5	80	10	7	-	-	-	32	11	100	12	10	-	-	-
6	42	8	12	3	17	3	10	4	20	4	6	3	6	31
7	9	3	12	3	-	-	4	1	9	3	4	2	-	-
9	30	6	44	5	4	-	12	4	100	9	2	-	-	-

	103	109	110	111	112	113	114	115	116	121	123	124	125	126
1	-	9	-	11	6	13	6	-	-	-	5	10	10	7
2	-	-	-	8	3	-	-	4	-	-	-	5	5	22
3	-	9	5	13	5	12	5	6	-	-	7	9	26	11
4	-	8	3	6	4	5	2	3	-	-	5	88	37	8
5	-	20	7	8	5	-	-	4	-	8	9	24	13	5
6	4	7	5	10	4	3	2	8	3	3	43	13	55	11
7	-	3	4	3	-	3	32	5	-	-	-	2	4	2
9	-	5	2	5	3	5	1	2	-	1	2	20	30	8

	127	128	129	130	131	135	137	138	139	140	141	142	143	144
1	-	-	-	-	-	-	-	-	9	-	-	-	-	-
2	4	1	100	10	2	-	-	-	-	-	-	-	-	-
3	7	3	8	-	-	-	7	3	7	8	4	5	3	-
4	5	6	8	-	-	-	4	2	5	15	7	3	3	-
5	4	3	11	-	-	6	10	7	12	10	6	-	-	-
6	6	3	32	6	3	-	6	3	8	5	3	2	5	27
7	4	2	12	2	-	-	2	1	1	1	1	100	18	2
9	22	80	11	11	-	-	15	4	6	23	5	3	3	-

Table 9. Continued

	145	147	149	150	151	152	153	154	155	156	157	158	159	163
1	-	-	-	7	-	-	-	-	-	-	-	-	-	-
2	-	11	2	-	-	-	-	-	4	-	-	-	-	-
3	-	-	8	-	5	5	6	20	5	5	-	-	-	-
4	-	-	5	3	4	-	3	3	2	-	56	9	-	-
5	-	-	6	3	9	4	7	8	4	-	-	-	-	5
6	3	-	7	3	4	8	-	-	-	-	4	-	-	-
7	-	-	4	-	-	-	-	-	7	4	2	-	-	-
9	-	-	2	1	2	1	3	3	66	13	38	14	4	-

	165	166	167	168	169	172	175	177	179	180	181	182	185	193
1	-	6	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	4	26	7	-	-	-	-	-	-	-	-	-	100	-
4	4	4	17	4	-	-	-	-	-	-	-	-	-	5
5	24	18	60	12	4	-	-	-	11	6	6	4	-	15
6	3	3	33	8	-	7	-	-	-	-	-	-	-	-
7	3	1	8	-	-	-	-	-	-	-	-	-	-	1
9	3	3	11	2	1	-	1	1	1	-	-	-	-	2

Table 9. Continued

	194	199	200	204	207	208	214	221	222	227	236	237	239	246	248
1	-	-	-	-	-	-	-	-	-	-	-	-	5	-	5
2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	2	12	3	-	6	-	-	4	-	-	3	6	-	-	-
5	7	-	-	-	17	5	-	19	5	-	15	5	-	-	-
6	-	100	15	-	2	-	-	3	-	-	2	3	-	-	-
7	-	1	-	-	1	-	1	1	-	1	1	-	-	1	-
9	-	-	-	3	4	1	-	3	-	-	2	-	-	-	-

	249	250	253	254	264	282	283
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	-	6	-	-	-	-	-
4	5	-	-	-	-	-	-
5	35	7	-	-	16	-	-
6	3	-	-	-	-	-	-
7	-	1	-	1	-	-	-
9	4	1	8	2	3	9	2

This residue was then dissolved in ether and the mixture was filtered; the ether was then evaporated to yield a pale yellow solid having a faint odor of pyridine. The material was washed by trituration with two 25-ml portions of cold 1 *N* hydrochloric acid and then with an equal volume of water. The residue was dissolved in ether and dried. Evaporation of the ether yielded a pale yellow gum [16 mg, R_F values of 0.0, 0.22, 0.33, 0.57, and 0.81 in E-*t*BuOH (45-2)]. Gas chromatography of the product at different temperatures and programming conditions failed to separate the aggregation of undefined peaks. A check of the products by GC-MS failed to reveal any bromo compounds present in the products. At least one of the peaks exhibited characteristics like that of didehydro X (Plate 14).

Nitric Acid Oxidation of Compound X

A sample (18 mg) of compound X was dissolved in 25 ml of 15 *M* nitric acid, and the solution was allowed to stand in a bath of ice for 1 hr. The solution was then maintained at room temperature for 1 hr, and then heated in a water bath at 60° for 1 hr. The reaction mixture was then maintained at steam bath temperature for 1 hr.

When the solution had cooled, the *p*H was adjusted to 1.5 using 20% aqueous sodium hydroxide. The solution was then distilled to near dryness. Water (50 ml) was then added, and the distillation was continued to dryness.

The distillate was made basic (*p*H 11), and the solvents were evaporated. The residue was dissolved in 3 ml of water, and the *p*H was adjusted to 5.0 with concentrated hydrochloric acid. To this solution,

130 mg of *p*-phenylphenacyl bromide and 10 ml of methanol were added. The mixture was boiled under reflux for 2.5 hr; then, after cooling, the mixture was diluted with 10 ml of water and extracted with three 15-ml portions of chloroform. The chloroform solution was then dried. The chloroform was evaporated to yield 0.065 g of yellow crystals.

Gas chromatography (5 ft 3% SE-30, CT 200°) yielded peaks with t_R of 2.6, 5.5, 8.0, and 10.9; standard esters (*p*-phenylphenacyl acetate, propionate, isobutyrate, and butyrate) had t_R of 7.5, 10.2, 11.7, and 14.0, using the same conditions. An extrapolation on the graph of log t_R vs. number of carbons for these standards indicated that *p*-phenylphenacyl hexanoate would have a t_R of 21. The recorder was allowed to run past this point for the products.

The material left in the distilling flask was dissolved in 35 ml of water, and this solution was continuously extracted with chloroform for 72 hr. The chloroform was evaporated, yielding 10 mg of a pale yellow syrup.

The syrup was dissolved in 5 ml of methanol, and 5 ml of 2,2-dimethoxypropane and 1 drop of concentrated hydrochloric acid were added. After standing for 8 hr, the solvents were evaporated, the residue was dissolved in 25 ml of ether, and the ethereal solution was washed with 5 ml of 5% aqueous sodium bicarbonate and 5 ml of water. After the solution was dried, evaporation of the ether gave 17 mg of a pale yellow oil.

Programmed temperature gas chromatography (5 ft 3% SE-30, 1pr 8°/min, starting temperature 130°) gave T_R of 135°, 140°, 150°, 155°, 160°, 172°, 177°, 183°, 187°, 190°, 200°, and 207°.

The oxidation was repeated as described, using 54 mg of compound X and 25 ml of 15 *M* nitric acid. The product was not distilled, but was neutralized to *p*H 2.5 with 20% sodium hydroxide,* and this solution was continuously extracted with chloroform for 64 hr. Evaporation of the chloroform yielded 19 mg of a yellow oil (overripe orange odor).

This material was then treated with 2,2-dimethoxypropane as described previously. Results of the GC-MS analysis of this product are summarized in Table 10. For the analysis, a 12 ft 3% OV-17 column was used; the starting temperature was 150°, with 1pr of 6°/min. Some 18 components were eluted; the intensity of several of the peaks was too small to obtain mass spectra therefrom. The peaks numbered 3 (13%, possibly complex) and 12 (60%) were the major fractions. An isothermal GC run (215°, 12 ft OV-17) indicated t_R of 2.0 and 9.0 for these peaks; standards dimethyl suberate, sebacate and tetradecanedioate had t_R of 1.3, 2.8, and 12.4 under the same conditions.

The mass spectrum of peak 12 is also given as Plate 8.

An attempt was made to determine the mass spectrum of peak 12 at lower electron energies. At 25 eV the relative intensities of the ions were approximately the same as at 70 eV. Limitations of the instrument precluded obtaining spectra at lower electron energies.

The oxidation was repeated, using 41 mg of compound X : 28 mg of a pale yellow gum was obtained.

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*The *p*H was inadvertently taken past 5; at this point the solution became yellow. Upon reacidification to *p*H 2.5 with a few drops of concentrated sulfuric acid, the solution became colorless again.

The gum was dissolved in 1.5 ml of dry pyridine (solution became more intensely yellow), and 1 ml of *N,O*-bis-(trimethylsilyl)acetamide was added. The solution was then heated on a water bath at 60-70° for 3 hr. After cooling, the solution was used for GC-MS analysis (12 ft 3% OV-17). Four peaks were observed: at a CT of 175°, a peak was eluted at t_R 3.0 (4%) the temperature was then increased to 210°; peaks were observed at t_R 7.7 (11%) and 9.8 (11%). After the CT was raised to 225°, the major peak (74%) was observed at t_R 12.6. This procedure was used for GC-MS; the mass spectra are given in Plates 9, 10, 11, and 12.

Preparation and Purification of Didehydro X

When compound X was impure, three spots were observed in the tlc system E-*t*BuOH (99:1) at R_F values of 0.04, 0.30, and 0.55 (all uv visible). Upon multiple development, the two spots at higher R_F values appeared to increase in intensity, while the spot at the lower R_F value decreased.

A sample of X was spotted on a preparative plate (75 mm) and developed in E-*t*BuOH (200:9). Each zone collected gave the identical three spots described above when chromatographed in E-*t*BuOH (99:1).

Another preparative thin layer was accomplished using ether-methanol (150:9) as eluent. The two zones observed (R_F values of *ca.* 0.50 and *ca.* 0.60) by uv light were submitted for tlc-MS. Each mass spectrum exhibited ions at highest mass of *m/e* 264 (36 mass units less than the mw of compound X).

In order to determine whether these substances could arise from compound X, 38 mg of crystalline X was stirred for 1 hr at room

Table 10. Mass Spectra of Products from Nitric Acid Oxidation of Compound X

Components in Order of Elution	Relative Abundance of Peaks at *m/e* =											
	39	40	41	42	43	44	45	53	54	55	56	57
2	5	3	15	2	14	6	-	5	-	17	5	8
3	4	-	15	3	9	4	2	3	1	13	17	12
4	5	3	18	5	11	5	-	5	-	15	33	11
5	20	10	42	13	44	18	10	12	10	43	30	34
6	21	14	50	16	50	26	10	10	-	44	20	33
7	20	10	40	13	40	18	5	10	5	35	21	63
8	13	6	32	7	32	10	-	5	-	23	15	100
12	18	7	58	12	98	14	3	7	3	40	20	20
	58	59	60	65	67	68	69	70	71	72	73	74
2	-	12	-	9	12	4	35	6	12	-	9	-
3	2	1	-	-	5	2	45	10	10	19	4	-
4	-	-	-	-	5	5	100	24	8	-	-	-
6	6	20	5	-	15	14	100	25	21	14	14	5
7	16	-	21	-	15	10	70	20	17	10	14	13
8	10	16	-	-	17	14	46	18	25	15	10	5
9	9	6	-	-	7	5	40	11	27	15	7	-
12	5	55	-	2	12	5	70	18	18	3	-	-
	83	84	85	86	87	95	96	97	98	99	100	101
2	56	9	9	-	-	24	9	13	4	15	-	-
3	10	5	100	12	1	4	1	3	1	8	1	2
4	16	10	5	-	-	5	3	5	5	6	-	-
6	100	20	90	10	14	20	25	23	11	20	10	16
7	100	50	100	10	15	22	16	16	10	12	10	-
8	40	20	100	13	4	23	15	19	10	17	10	10
9	-	-	85	12	6	15	10	11	5	10	5	-
12	44	20	100	10	2	11	6	56	29	12	77	10

Table 10. Continued

	102	103	109	110	111	112	113	115	116	123	124	125
2	-	-	19	4	15	4	5	23	4	6	5	25
3	-	-	3	-	3	-	2	-	-	2	-	-
4	-	-	-	-	9	4	-	3	-	-	-	-
6	10	10	20	10	15	10	25	72	15	15	11	34
7	-	19	-	18	10	-	15	40	-	14	-	17
8	-	-	20	9	17	10	20	20	-	12	5	15
9	-	-	11	-	10	-	10	7	-	9	-	5
12	-	2	5	4	24	5	22	-	-	2	2	8

	126	127	128	129	130	131	132	135	136	137	138	139
2	8	9	4	3	-	-	-	-	-	-	-	6
3	1	12	1	-	-	-	1	-	-	-	-	-
4	54	8	-	-	-	-	-	-	-	-	-	-
6	19	15	25	15	6	-	-	5	7	15	7	6
7	20	40	10	11	9	10	-	-	-	-	-	-
8	18	18	35	10	10	-	-	-	-	13	13	10
9	11	7	25	5	4	-	-	-	-	-	5	5
12	6	15	22	-	-	-	-	1	-	1	-	3

	140	141	142	143	144	145	149	151	152	153	154	155
2	4	13	4	18	4	-	-	11	5	7	4	9
3	-	-	12	1	3	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
6	-	13	15	15	10	10	10	-	39	15	-	19
7	-	-	-	25	22	-	-	-	-	-	-	-
8	7	36	10	20	14	-	-	-	-	14	9	17
9	-	22	-	-	-	-	-	-	-	5	-	5
12	-	1	-	-	-	-	1	1	-	10	1	1

Table 10. Continued

	156	157	158	159	165	166	167	168	169	172	173	175
2	-	-	43	8	-	-	22	6	6	-	-	-
3	2	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
6	13	9	-	-	12	-	12	-	-	21	35	-
7	10	10	-	-	-	-	15	-	-	-	-	-
8	85	20	-	18	-	-	-	-	-	-	-	25
9	52	13	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	28	10	4	-	-	-

	183	185	195	197	199	200	201	211	212	213	219	223
2	-	-	7	-	38	7	3	100	15	7	-	-
3	-	-	-	-	t	-	-	4	-	-	t	-
4	-	-	-	-	-	-	-	8	-	-	-	-
6	10	22	-	10	-	-	-	14	9	8	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	11
9	-	-	-	-	-	-	-	-	-	-	-	-
12	-	13	5	-	-	-	-	-	-	8	-	-

	226	227	230	238	246	255	256	257
2	13	4	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	3	-	-	-	-	-	-	-
6	-	-	11	-	5	10	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	14	-	15	-	-
9	-	-	-	-	-	-	-	-
12		-	-	-	-	36	6	1

temperature with 1 ml of concentrated hydrochloric acid. At the end of this time, oily droplets were observed floating on the liquid. The mixture was diluted with water; an insoluble, sticky material precipitated on the sides of the flask. The acid solution was decanted, and the residue was rinsed twice with distilled water.

The product was then dissolved in ether and dried. Evaporation of the ether yielded 17 mg of product. An ir spectrum (film) of this material exhibited absorptions at 3420 (br), 2960, 2930, 2870, 1745 (br), 1673, and 1465 k, among others. (See Plate 13.)

Thin-layer chromatography of this material (E-*t*BuOH, 40:1) resulted in R_F values of 0.1 (faint), 0.73 (very intense), and 0.93 (faint) (detection, uv light). A preparative tlc procedure was done using this material; the most intense zone was submitted for tlc-MS. The mass spectrum obtained is given as Plate 14. A 60 MHz nmr spectrum (deuterochloroform solvent) of the remaining material exhibited absorptions at τ 5.10 (d, J = 4), 5.57 (d, J = 4), 5.99 (d, J = 4), 6.35 (br abs), 7.20 (br abs), 7.80 (q, J = 7.5), 8.30 (br abs), 8.65 (many abs), 8.85, 8.96, 9.0, 9.05, and 9.15.

A more efficient method of obtaining didehydro X was sought. A sample of 202 mg of compound X (mp 98-101°) was dissolved in 12 ml of dioxane, and 1.25 ml of concentrated hydrochloric acid was added. After 20 hr, 12 ml of 5% sodium bicarbonate was added to the solution, and neutralization (*p*H 7) was completed with solid sodium bicarbonate. The solution was then extracted with three 25-ml portions of ether. The ether-dioxane solution was dried, and the solvents were evaporated.

This material was then purified by preparation tlc [solvent, E-*t*BuOH (200:9)]. The zone (R_f *ca.* 0.60) was processed in the usual manner. A total of 53 mg of product was obtained. A 60 MHz nmr spectrum was obtained which was very similar to the one obtained of the product from the preceding reaction. A 100 MHz nmr spectrum was also obtained of this material. It is given as Plate 15. A uv spectrum of this material exhibited λ_{max}^{EtOH} 237 nm (ϵ 9000). An ir spectrum (chloroform solution) exhibited absorptions at 3570, 3330 (br), 2930, 2850, 1750, 1675, and 1465 k, among others.

A high-resolution mass spectrum was obtained; this is given as Table 11.

It was later found that approximately equal quantities of dioxane and hydrochloric acid used in the preparation of didehydro X would result in a greater yield of the product. In one instance, 167 mg of compound X, 2 ml of dioxane, and 2 ml of hydrochloric acid were used, yielding 137 mg of crude product. This is contrasted with a preparation utilizing 143 mg of compound X, 10 ml of dioxane, and 1 ml of concentrated hydrochloric acid, in which 65 mg of crude product was obtained.

Attempted Preparation of Didehydro X by Other Methods

A test tube containing 15 mg of compound X was heated in a Woods metal bath to 210°, and this temperature was maintained for 2 hr. The tube was then cooled, weighed, and the product was obtained by rinsing the tube with two 0.5-ml portions of ether. The tube was then reweighed to determine the amount of product. A yield of 11 mg was obtained.

Table 11. High Resolution Mass Spectrum of Didehydro X

CALCULATED MASS	ERR	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
NO COMP CALC						273.9711	5	++++++++++++
264.1724	.12	16/0	24	0	3	264.1726	15	++++++++++++++
NO COMP CALC						262.9831	6	++++++++++++
NO COMP CALC						250.1431	8	++++++++++++
249.1490	-2.92	15/0	21	0	3	249.1461	20	++++++++++++++
249.1445	1.55	14/1	20	0	3			
236.1775	-1.31	15/0	24	0	2	236.1762	21	++++++++++++++
236.1731	3.14	14/1	23	0	2			
221.1541	-2.86	14/0	21	0	2	221.1512	17	++++++++++++++
221.1496	1.58	13/1	20	0	2			
NO COMP CALC						214.0261	7	++++++++++++
207.1384	1.67	13/0	19	0	2	207.1401	16	++++++++++++++
207.1021	.42	12/0	15	0	3	207.1025	13	++++++++++++++
193.0864	- .64	11/0	13	0	3	193.0858	17	++++++++++++++
193.0819	3.87	10/1	12	0	3			
179.0708	3.41	10/0	11	0	3	179.0742	7	++++++++++++
NO COMP CALC						173.9857	6	++++++++++++
170.0003	.24	10/0	2	0	3	170.0006	5	++++++++++++
167.1435	.97	11/0	19	0	1	167.1445	19	++++++++++++++
167.0708	- .61	9/0	11	0	3	167.0702	5	++++++++++++
167.0663	3.87	8/1	10	0	3			
166.0629	- .97	9/0	10	0	3	166.0620	14	++++++++++++++
166.0584	3.54	8/1	9	0	3			
165.0551	1.12	9/0	9	0	3	165.0562	16	++++++++++++++
153.0551	.76	8/0	9	0	3	153.0559	9	++++++++++++

CALCULATED MASS	ERR	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
151.0758	.79	9/0	11	0	2	151.0766	5	++++++++++++
137.0602	.06	8/0	9	0	2	137.0603	9	++++++++++++
124.1251	- .68	9/0	16	0	0	124.1245	18	+++++++++++++++
124.1207	3.79	8/1	15	0	0			
109.1017	- .27	8/0	13	0	0	109.1014	14	+++++++++++++++
109.0653	1.32	7/0	9	0	1	109.0666	5	++++++++++++
109.0653	-3.31	7/0	9	0	1	109.0619	5	++++++++++++
109.0608	1.15	6/1	8	0	1			
NO COMP CALC						100.9366	9	++++++++++++
97.0653	1.08	6/0	9	0	1	97.0664	11	++++++++++++
97.0289	- .13	5/0	5	0	2	97.0288	18	+++++++++++++++
95.0860	.61	7/0	11	0	0	95.0866	17	+++++++++++++++
95.0496	.50	6/0	7	0	1	95.0501	13	+++++++++++++++
93.0704	1.48	7/0	9	0	0	93.0719	8	++++++++++++
91.0547	.65	7/0	7	0	0	91.0554	6	++++++++++++
83.0860	- .36	6/0	11	0	0	83.0856	16	+++++++++++++++
83.0496	-1.08	5/0	7	0	1	83.0485	14	+++++++++++++++
83.0451	3.38	4/1	6	0	1			
82.0782	.12	6/0	10	0	0	82.0783	5	++++++++++++
81.0704	- .71	6/0	9	0	0	81.0696	14	+++++++++++++++
81.0659	3.76	5/1	8	0	0			
79.0547	.41	6/0	7	0	0	79.0551	7	++++++++++++
79.0547	-3.49	6/0	7	0	0	79.0512	5	++++++++++++
79.0502	.99	5/1	6	0	0			
77.0391	.30	6/0	5	0	0	77.0394	8	++++++++++++

CALCULATED MASS	ERR	C12/13	H	N	O	MEASURED MASS	NO. PTS	INTENSITY
72.0575	-1.11	4/0	8	0	1	72.0564	10	++++++++++++
72.0530	3.37	3/1	7	0	1			
71.0860	- .36	5/0	11	0	0	71.0856	11	++++++++++++
69.0704	.01	5/0	9	0	0	69.0704	17	++++++++++++++
69.0340	- .57	4/0	5	0	1	69.0334	18	++++++++++++++
69.0295	3.89	3/1	4	0	1			
67.0547	.41	5/0	7	0	0	67.0551	18	++++++++++++++

LIMIT OF DATA

This material had R_F values of 0.0, 0.63, 0.74, and 0.82 in the system E-*t*BuOH (45:1). In the same system, didehydro X had an R_F value of 0.63. Using a 5 ft 3% SE-30 column with a starting CT of 130° and a 1pr of 10°/min, the material had t_R (relative area) of 12.2 (4), 13.7 (23), and 19.2 (73). A study by GC-MS revealed that the peaks at t_R 12.2 and 13.7 had mass spectra like that of didehydro X; the spectrum of the peak at t_R 19.2 is given as Plate 16. All spectra are given as Table 12.

In another experiment, 55 mg of compound X (mp 101-104°) was dissolved in 3 ml of formic acid. To this solution 3 ml of concentrated hydrochloric acid was added. The solution was covered loosely and allowed to stand overnight. After this time, neutralization was begun by adding 20 ml of 5% sodium bicarbonate, followed by a considerable quantity of solid sodium carbonate. By the time a *p*H of 6 was reached, material had precipitated to the sides of the flask. The aqueous solution was then decanted from the precipitate and the precipitate was rinsed with two flask volumes of water. The solid material was then scraped from the flask and filtered.

An attempt was made to purify the material by preparative tlc; no separation took place. The material was eluted from the silica in the usual manner, yielding 10 mg of a colorless oil. The oil crystallized after having been scratched. The resulting semicrystalline material had a mp of 55-70°. A mass spectrum was obtained which had features common to the spectra of both monodehydro X and didehydro X.

Table 12. Mass Spectra of Products Resulting from Heating Compound X

Compound in Order of Elution	Relative Abundance at *m/e* =											
	39	40	41	42	43	44	51	53	54	55	56	57
1	15	8	55	10	63	10	10	10	-	51	17	20
2	3	-	10	-	6	-	-	5	2	14	2	3
3	0	-	24	-	19	-	-	7	-	21	4	12
	65	66	67	68	69	70	71	72	77	79	80	81
1	8	8	26	10	100	17	57	12	13	15	-	26
2	2	-	9	3	38	5	4	-	4	4	3	10
3	5	-	12	5	52	8	18	5	8	8	5	13
	82	83	84	85	86	91	93	94	95	96	97	98
1	18	45	12	24	-	10	12	10	25	16	70	10
2	12	40	4	3	-	4	4	3	20	7	64	6
3	10	40	2	20	5	-	-	-	20	5	87	10
	99	105	107	108	109	110	111	112	121	122	123	124
1	22	-	10	8	20	12	17	-	10	6	21	15
2	5	3	6	3	15	5	4	2	12	2	7	18
3	14	-	8	-	17	-	9	-	-	-	10	25
	125	126	127	128	129	135	136	137	138	139	140	141
1	20	24	3	10	-	11	-	30	14	10	-	-
2	7	5	3	-	-	7	3	13	8	14	12	5
3	22	11	19	52	11	-	-	22	11	20	27	23
	142	149	150	151	152	153	154	155	156	157	158	159
1	-	-	-	19	10	19	7	50	7	-	-	-
2	-	5	3	14	5	10	11	3	-	-	-	-
3	8	7	-	12	8	13	15	100	20	36	16	7
	161	163	164	165	166	167	168	169	175	177	178	179
1	-	-	-	25	20	27	-	-	-	24	14	41
2	2	12	3	44	32	100	18	4	4	6	4	20
3	-	13	-	31	21	70	15	7	-	-	-	10

Table 12. Continued

	180	181	182	183	184	189	191	193	194	195	203	204
1	-	-	-	-	-	-	-	40	14	5	-	-
2	12	10	8	5	-	4	4	38	13	5	6	4
3	-	17	10	11	7	-	-	27	10	10	-	-

	205	206	207	208	210	221	222	235	236	237	238	249
1	-	-	14	7	-	-	-	-	-	-	-	32
2	-	-	47	13	-	52	12	4	47	11	3	92
3	-	-	30	10	7	30	10	5	27	9	-	52

	250	251	253	254	264	265	282	283
1	10	-	-	-	34	6	-	-
2	18	3	-	-	25	6	-	-
3	11	-	18	12	18	-	35	12

The material had R_F values of 0.63 and 0.81 in the system E-*t*BuOH (45:1). In this system, didehydro X had an R_F value of 0.63. An attempt was again made to purify the product by preparative tlc. A yield of 3 mg of semicrystalline product was obtained, which had R_F values of 0.67 and 0.77 in the system E-*t*BuOH (45:1). A mass spectrum of this product was identical to the one obtained from the initial product.

Gas Chromatography of Didehydro X

When a sample of freshly prepared didehydro X was injected at 237° CT and 210° IT (5 ft 3% SE-30), a single symmetrical peak was observed at t_R 5.2 min. When programming at a lpr of 10°/min, starting temperature 175°, one peak was observed at T_R 251° for didehydro X. The IT was raised to 250° in a subsequent injection; a small peak (*ca.* 5%) appeared at a T_R of 15° less than that of didehydro X. The IT was then raised to 300° and the injection was repeated: the small peak increased in size (*ca.* 10%).

The sample of didehydro X (in methanol solution) used for the previous experiment was allowed to stand at room temperature for a week, and a second experiment was carried out (starting CT, 100°; IT, 225°, lpr 10°/min). The major peak was observed with a T_R of 235°; no peak preceded it, but a small peak followed at T_R 248°. The injection was repeated with an IT of 325°. A peak (*ca.* 11%) was then observed at T_R 208° preceding the major peak at T_R 232°. A small peak followed the major peak at T_R 242°.

Hydrogenation of Didehydro X

A sample of 16 mg of didehydro X was dissolved in 15 ml of methanol and placed in a pressure bottle. To this solution was added 20 mg of 5% platinum on carbon dispersed in 5 ml of methanol. The solution was hydrogenated at 10 psig for 30 hr.

The solution was then filtered through a Celite mat (aspirator vacuum). The mat was washed with several 1-ml portions of methanol. The solvent was then evaporated from the filtrate. The residue was dissolved in 1 ml of methanol for GC-MS studies.

Using a 5 ft 3% SE-30 column, 1pr 10°/min, starting temperature 100°, peaks were observed at T_R (relative amount) 129° (43%), 202° (12%), and 214° (45%). The mass spectral data corresponding to these peaks are given as Table 13.

Permanganate Oxidation of Didehydro X

A sample of 40 mg (1.5 meq) of didehydro X, prepared as previously described, was dissolved in 1.5 ml of pyridine. A solution of *ca.* 2.5% aqueous potassium permanganate was added dropwise. A total of 3.2 ml (2.0 meq) of the solution was added; the flask was then warmed on a water bath at 60°. After 20 min of heating, the purple color was discharged. Another meq was then added and heating was continued for 1 hr. A light purple coloration persisted after this time, and was discharged with sulfur dioxide.

The solution was then filtered (aspirator vacuum) through a Celite mat. The flask and the mat were washed with distilled water and the washings were added to the filtrate. The mat and the flask were

Table 13. Mass Spectra of Products from Hydrogenation of Didehydro X

Components in Order of Elution	Relative Abundance at *m/e* =											
	39	40	41	42	43	44	45	53	55	56	57	65
1	-	-	4	-	-	-	-	-	-	-	-	-
2	2	2	8	7	7	3	-	2	12	4	8	1
3	4	4	11	-	7	9	3	5	18	-	-	-
	67	68	69	70	71	72	73	77	78	79	80	81
1	3	-	-	-	-	-	11	14	5	41	8	9
2	4	2	15	12	7	20	3	1	-	2	-	4
3	10	-	27	6	4	-	-	6	4	7	2	9
	82	83	84	85	86	88	91	92	93	94	95	96
1	-	-	-	-	3	4	40	17	41	49	9	3
2	3	14	5	100	10	-	8	-	2	-	3	1
3	5	29	4	9	-	-	8	-	6	4	18	6
	97	98	99	101	102	103	104	105	106	107	108	109
1	4	3	8	73	9	4	8	20	23	10	7	5
2	5	-	11	-	-	-	-	6	8	8	-	2
3	51	6	7	-	-	-	-	6	-	9	5	14
	110	111	112	113	114	115	117	119	120	121	122	123
1	-	4	6	-	7	3	5	23	77	100	22	16
2	1	5	17	8	-	2	2	1	-	1	-	3
3	5	8	4	4	-	-	-	-	-	8	4	10
	124	125	126	127	128	129	130	131	133	135	136	137
1	6	11	-	13	5	3	8	3	7	3	-	8
2	-	4	9	3	-	-	-	-	-	-	-	3
3	15	10	7	5	5	4	-	-	-	9	4	14
	138	139	140	141	142	143	145	146	147	148	149	150
1	4	5	11	2	-	-	-	-	11	83	46	28
2	-	-	-	-	-	-	-	-	4	-	-	-
3	7	14	14	7	4	8	5	4	6	-	9	6

Table 13. Continued

	151	152	153	154	155	156	157	158	159	161	163	164
1	16	12	11	-	-	-	-	-	-	-	-	-
2	-	-	-	-	4	37	6	1	-	-	-	-
3	15	14	13	14	20	9	14	9	5	6	15	5

	165	166	167	168	169	170	171	172	173	174	175	177
1	9	8	3	-	-	-	-	-	-	-	-	-
2	2	2	2	7	4	4	-	3	-	3	4	-
3	44	32	100	20	10	7	10	-	5	4	5	10

	178	179	180	181	182	183	184	185	186	187	188	189
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	2	15	5	4	-	-	-
3	7	29	7	14	15	8	8	7	15	6	9	10

	190	191	192	193	194	195	196	197	200	203	204	205
1	-	-	-	-	12	2	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	6	10	7	44	16	11	5	10	7	12	7	7

	206	207	208	209	210	211	212	213	215	220	221	222
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	4	1	11	-	7	4	-
3	5	55	19	7	10	8	4	6	4	-	59	15

	223	227	230	231	235	236	237	238	239	241	242	248
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	1	1	-	-	-	-	-	-	4	1	2
3	7	9	-	7	9	53	15	7	7	-	-	-

	249	250	251	253	254	264	265	282	283	284
1	-	-	-	-	-	-	-	-	-	-
2	4	-	-	-	-	-	-	4	-	4
3	100	16	7	30	10	24	7	39	9	-

washed with pyridine and chloroform; the chloroform was evaporated and the residue was added to the filtrate. The combined washings were made acidic (*p*H 1.5) with concentrated hydrochloric acid.

The acidic solution was then vacuum distilled using a water bath at 70°. The receiver was cooled in ice. The distillation was continued until a small amount of material remained in the flask; 30 ml of water was added and the distillation was continued to dryness.

The distillate was made basic (*p*H 11) with 20% aqueous sodium hydroxide. The solvents were then evaporated, and the residue was dissolved in 2 ml of water. The *p*H of this solution was adjusted to 5 with dilute hydrochloric acid.

To this solution, 107 mg of recrystallized *p*-phenylphenacyl bromide and 10 ml of methanol were added. The mixture was boiled under reflux for 2.5 hr. When the mixture had cooled, it was diluted with 20 ml of water and extracted with three 10-ml portions of chloroform. The solution was dried with sodium sulfate and the solvents were evaporated. A yield of 78 mg of crystalline material was obtained.

At a CT of 195° (5' 3% SE-30), the sample had t_R of 2.4, 5.0, 6.9, 8.1, and 11.0; a standard mixture of *p*-phenylphenacyl acetate, propionate, isobutyrate, and butyrate had t_R of 8.1, 11.3, 13.1, and 16.0. The products from a blank reaction exhibited peaks at t_R 2.2, 4.8, and 6.3.

The pale yellow nonvolatile material remaining in the distilling flask was then diluted with 25 ml of water. The solution was continuously extracted with chloroform for 48 hr. The chloroform solution was

then dried (sodium sulfate), and the chloroform was evaporated to yield 71 mg of a pale yellow, semicrystalline material with a faint odor of pyridine.

This material was dissolved in 5 ml of methanol and treated with 5 ml of 2,2-dimethoxypropane and one drop of concentrated hydrochloric acid. The solution was allowed to stand for 4 hr, and then processed as previously described. The resulting material gave the following GC results: in a programmed run (starting temperature 100°, 1pr 10°/min) peaks were observed at T_R of 115, 134°, 160°, 165° (not well separated),174°, 200°, 210°; several small peaks were observed beyond 210°.

Another oxidation was carried out: 137 mg of didehydro X was dissolved in 10 ml of acetone, and a 2% aqueous potassium permanganate solution was added until the purple color could be seen distinctly. A blank solution of acetone was also used. At room temperature, the blank solution retained the purple coloration (no manganese dioxide was observed), while the solution containing didehydro X became red.

The solution was then heated on the water bath at 60° for 20 min; the purple color was then not present. Another 5 ml of the permanganate solution was then added and the mixture was heated for 30 min. The blank solution was also oxidized at this rate by the permanganate solution. This step was repeated three times.

The solution was then filtered, and the mat was washed with distilled water. The acetone was evaporated from the solution, which was then made acidic (*p*H 1.5) with concentrated sulfuric acid. The

acidic solution was continuously extracted for 48 hr with chloroform. The solution was then dried, and the chloroform was evaporated to yield 138 mg of residue.

This material was then dissolved in methanol and treated with 2,2-dimethoxypropane and concentrated hydrochloric acid as previously described. The products were subjected to a programmed GC run. The resultant chromatogram was very much like that obtained for the non-volatile products from the previous oxidation.

A series of GC and GC-MS studies were carried out; the gas chromatographic data are given in Table 14. The mass spectral data for these four compounds are given as Table 15.

Table 14. Results of GC Studies of Products from Permanganate Oxidation of Didehydro X and Some Known Compounds

(CT (12'OV-17)	123°	145°	215°
	t_k	t_k	t_k
Peak 1	11.9	4.8	0.7
2	16.0	6.1	1.6
3		13.2	2.5
4			7.0
dimethyl succinate	3.2		
dimethyl 2,2-diethyl malonate	5.6		
dimethyl adipate	12.4	5.4	
dimethyl 2,5-dimethyl adipate		6.7	
dimethyl suberate		15.3	1.6
dimethyl sebacate			3.3
dimethyl tetradecanedioate			13.3

Table 15. Mass Spectra of Products from Permanganate Oxidation of Didehydro X

Component in Order of Elution	Relative Abundance at *m/e* =											
	39	40	41	42	43	44	45	53	54	55	56	57
1	40	19	100	26	35	19	-	-	-	24	33	11
2	13	5	32	10	27	10	6	4	2	14	12	13
3	13	-	25	6	11	-	-	1	-	19	89	24
4	8	2	23	4	12	4	2	4	2	16	12	15
	58	59	65	67	68	69	70	71	72	73	74	77
1	-	-	-	-	-	68	49	12	-	-	-	-
2	2	9	-	3	3	14	36	13	5	6	2	-
3	-	-	-	-	-	22	16	14	-	-	-	-
4	3	-	2	7	3	37	9	12	26	4	-	2
	83	84	85	86	87	95	97	98	99	100	101	102
1	-	-	-	-	-	-	-	-	19	-	-	25
2	-	-	-	-	18	-	-	-	13	-	5	100
3	30	9	25	-	-	10	5	15	100	16	15	-
4	9	8	100	10	2	2	2	2	5	1	1	2
	103	111	113	114	124	126	127	131	137	141		
1	-	-	-	-	-	15	-	-	-	-		
2	7	-	-	-	-	-	-	5	-	-		
3	-	-	100	11	1	-	-	-	1	-		
4	-	1	1	1	-	4	-	-	-	2		

CHAPTER III

DISCUSSION OF RESULTS

The purpose of this research was to investigate the chemistry of antibiotic X-5108 in order to gain information about its structure. This was done by employing degradative reactions in the hope that identifiable molecules would be produced.

Several reactions that were investigated using the antibiotic, received as the sodium salt, indicated that many impurities were present. Column chromatography was then employed to purify the material. Silicic acid was found to be unsatisfactory, and various types of Sephadex were tried. Sephadex G-15 was the most successful in separating the bright yellow antibiotic from a brown-gold impurity, but impurities remained.

It was found by biological assay that the bright yellow purified antibiotic had a biological activity slightly higher than that of the unpurified material, and that the brown-gold impurity had a biological activity significantly lower than the unpurified X-5108.

The antibiotic, after being converted to its acidic form, was then washed with hexane or precipitated from chloroform solution with hexane. Evaporation of the hexane yielded 8-10% by weight of hexane-soluble impurities.

It was thought that these impurities contained fatty acids; therefore, the hexane-soluble material was treated with

2,2-dimethoxypropane to convert any acidic materials to their methyl esters for gas chromatography. Comparison of these retention times with those of standard fatty acid methyl esters indicated the presence of the methyl esters of lauric, myristic, pentadecanoic, palmitic, heptadecanoic, stearic, and arachidonic acids. Methyl palmitate was the most abundant component of the mixture.

The purified X-5108 (H^+) gave only the R_F value of 0.65 in the system B-A-W (4:1:5), but in the system chloroform-methanol (45:5), two spots were observed; when each spot was eluted, re-spotted, and developed in the same solvent system, both spots were again observed. This seemed to indicate that a type of equilibration was taking place during the development of the chromatogram.

The phenomenon of two (or more) spots occurring from a single compound is well documented.[22] Some of the probable causes are compound-adsorbent interaction, compound-eluent interactions, equilibration between two species, or interconversion between two species. Only the last two mentioned would seem applicable in this case.

The formula of X-5108 (H^+) had been proposed as $C_{38}H_{56}N_2O_{11}$.[1] However, analytical data obtained using the highly purified material indicated that the formula was $C_{41}H_{60}N_2O_{12}$. A substance having this formula would have a molecular weight of 773; two determinations of the molecular weight by osmometry gave an average value of 772.5, and two determinations of the neutralization equivalent gave an average value of 773.5. This would seem to substantiate the proposed formula.

Based upon this formula, there were about three saponifiable groups in the molecule as given by the average of two saponification equivalent determinations of 261.5. Active hydrogen analysis gave an average value of 0.99%, which would indicate about eight active hydrogens present.

An *O*-methyl analysis gave a value of 4.18%, indicating only one *O*-methyl group. The *N*-methyl analysis gave a value of 8.31%, which indicated two *N*-methyl groups. A *C*-methyl analysis gave a value of 7.05%, indicating at least four *C*-methyl groups.

From the analytical data it can be observed that, if four oxygens are present as ester or lactone functions and the acidic function is present as a carboxyl group, and one oxygen is present as an *O*-methyl group, then a maximum of five hydroxyl groups is possible. If, however, the acidic function is a phenolic group, a maximum of six hydroxyl groups would be possible. The active hydrogen analysis is high in either case.

The method of active hydrogen analysis employed was the Zerewitinoff method, which, by employing methyl magnesium iodide in a Grignard reaction, produces methane which is then measured.

Experiments have shown[23] that some esters containing *alpha* hydrogens are susceptible to Grignard reagent enolization. The *alpha* hydrogen is abstracted to produce methane as shown in Equation 1.

$$RR'CHCO_2R'' \xrightarrow{R'''M_gX} [RR'{=}C(-O)-OR'' \longleftrightarrow RR'C-C(=O)-OR'']^{-}M_gX^{+} + R'''H \quad (1)$$

Perhaps a reaction of this type produces the high active hydrogen analysis observed for this compound.

It is also possible that amide N-H protons are also contributing to the active hydrogen analysis.

The infrared spectrum of X-5108 (H^+) (Plate 1) confirmed the presence of hydroxyl groups with an absorption at 3400 k (br); strong, broad absorptions 1670-1520 k suggested unsaturation and possibly unsaturated carbonyl functions. Saturated ester, lactone, or ketone absorptions (1750-1705 k) did not appear.

The ultraviolet spectrum had λ_{max}^{EtOH} at 232 nm, log ϵ 4.79, and 320 nm (285 sh), log ϵ 4.41, indicating the presence of conjugated systems.[23]

The 60 MHz nmr spectrum of X-5108 (H^+) was very complex, as would be expected for a molecule having 60 protons; however, information could still be obtained from it.

The absorption centered at 9.1 τ (*ca.* 12 H), agrees with the value of four methyl groups found by *C*-methyl analysis. An absence of absorptions in the region 8.5-9.0 τ points to the absence of shielded methylene groups: any methylene groups present must be adjacent to unsaturated or electronegative groups.

The absorptions at 8.31 and 8.2 were possibly due to $CH_3-\overset{|}{\underset{|}{C}}=\overset{|}{\underset{|}{C}}-$, $CH_3-\overset{|}{\underset{|}{C}}-X$, (X = OH, OR, N) and/or C-H; the absorptions at 7.95 (4 H) could be due to $CH_3-\overset{|}{\underset{|}{C}}-X$, or $CH_3-\overset{|}{C}=O$, the absorption at 6.82 τ (5 H) could possibly be partly due to a $CH_3-N\diagup\diagdown$ function, or to $\diagup\diagdown CH-C=O$, while the absorption at 6.53 may be $-CH_2-O$, or CH_3-O. The absorption at

6.25 (3H) may be construed to be the methoxyl group indicated by the analytical data. Hydroxyl protons and exomethylene groups could give rise to the absorptions in the region 5.5-6.0 (7 H). The large number of absorptions in the region 3.4-4.6 τ (15 H), as well as the absorption centered at 2.55 τ (1 H), suggested the presence of protons attached to unsaturated or aromatic systems. Notably absent from the nmr spectrum was absorption at lower field than 2.0 τ.

When the temperature of the deuterochloroform solution of X-5108 (H^+) was raised, the only discernible change was in the complexity of the region 3.4-4.6 τ. This would seem to indicate that possible migrations and isomerizations were taking place that involved the double bonds. It is known that solutions of X-5108 are unstable to heat; the acidic form is more unstable than the sodium salt.

An nmr spectrum of X-5108 (Na^+) in a solution of deuterium oxide and acetone -d_6 was obtained, both at ambient temperature and at 80°. No change was observable in the spectrum.

A trimethylsilyl derivative of the antibiotic was prepared for the mass spectral analysis: it was hoped that a definitive molecular weight would be observed and possibly the number of hydroxyl groups would be established. The mass spectrometer which was employed, however, lost a large amount of its resolving power above *m/e* 700; therefore values of *m/e* stated must remain approximate. At *m/e ca.* 1219, a peak was observed. If this peak corresponded to the molecular ion, then a value of six trimethylsilyl groups (mass 73 each) could be calculated; hence, six hydroxyl groups could be present.

It was desired to obtain a crystal of the antibiotic for possible X-ray studies. The amorphous powders X-5108 (Na^+) and highly purified X-5108 (H^+) resisted crystallization; therefore an attempt was made to prepare the rubidium salt of X-5108.

This was accomplished by pouring a solution of highly purified X-5108 (H^+) through an IRC-50 ion-exchange column in the rubidium phase. The resulting material had a deeper color than X-5108 (Na^+) or X-5108 (H^+) and exhibited a different melting point from either.

The nmr spectrum of the material believed to be X-5108 (Rb^+) exhibited only very broad lines in a "mountain range" effect. One would predict some broadening due to the quadrupole moments of the two isotopes of rubidium present, but not to this extent: it might be inferred that some effect such as chelation might be taking place. Nevertheless, no crystals were obtained of this salt.

An anhydrous methanolysis was attempted in order to ascertain whether any sugars were present in the molecule. The reaction was expected to produce methyl glycosides, which would then be converted to acetyl derivatives for comparison with standards by gas chromatographic retention times. As a result of the reactions, no products with volatilities high enough to be eluted before octaacetylsucrose were produced.

Several hydrolyses of X-5108 were undertaken in the hope that a molecule of small to intermediate size would be produced. The nonvolatile products of these reactions, however, were exceedingly complex, and no compounds were identified. Several of the volatile compounds produced, however, were identified.

A vigorous basic hydrolysis using 6 *N* sodium hydroxide and reflux conditions was carried out. The reaction mixture was distilled into hydrochloric acid, and the distillate was evaporated for the studies. Ninhydrin indicated one spot, which was found to have a comparable R_F value to that of methylamine hydrochloride.

A vigorous acidic hydrolysis using 2 *N* sulfuric acid was then performed, and the reaction mixture was distilled to collect any volatile acids. The distillate was evaporated and treated with *p*-phenylphenacyl bromide to convert any acids present to their *p*-phenylphenacyl esters. Gas chromatography of the resulting products indicated no *p*-phenylphenacyl esters were present. This experiment was not strictly indicative that no volatile acids were produced, as the successful preparation of *p*-phenylphenacyl derivatives is highly dependent upon having the correct *p*H.

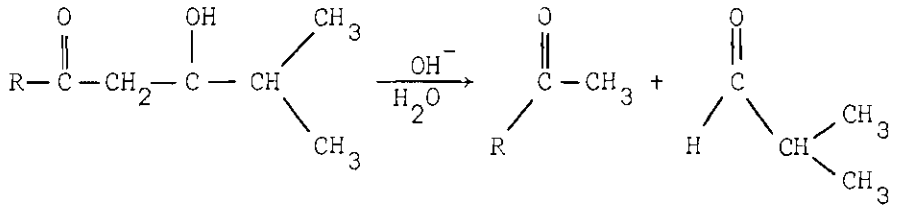
From the same acidic hydrolysis, the reaction mixture was then made basic and distilled into 6 *N* hydrochloric acid. Evaporation of the solvents gave a residue that had an R_F value identical to that of methylamine in several solvent systems.

An acidic hydrolysis was then carried out using hexane-washed X-5108 (H^+) and 2 *N* sulfuric acid under reflux conditions in order to investigate the nonvolatile products. These were found to be very complex by tlc.

A mild basic hydrolysis using hexane-washed X-5108 (H^+) and 0.1 *N* sodium hydroxide was then carried out. The volatile effluent of the reaction was trapped with 2,4 dinitrophenylhydrazine reagent, and the

resultant yellow needles were identified as isobutyraldehyde 2,4 dinitrophenylhydrazone by comparison of melting points and nmr spectra.

This would seem to indicate that a reverse aldol condensation was taking place during the reaction as indicated in Equation 2.



(2)

The reaction mixture from this mild basic hydrolysis was then neutralized and extracted with chloroform. The residue demonstrated several well-resolved GC peaks. It was desired to obtain GC-MS of these peaks; the sample was sent for this determination, but was lost in transit.

A basic hydrolysis was then carried out using highly purified X-5108 (H^+) and 6 *N* sodium hydroxide. It was hoped to isolate the methylamine hydrochloride from the distillate for mass spectrometry, but although the ninhydrin-positive material was observed to be present by tlc, isolation was not possible. Methylamine hydrochloride sublimes easily and is deliquescent, and these properties would make it difficult to isolate. An attempt was also made to obtain the mass spectrum by tlc-MS, but only the background from the silica gel HF_{254} plate was observed. (Samples from silica gel plates exhibit backgrounds that have a typical hydrocarbon distribution of ions, as well as silicone ions.)

The mixture in the distilling flask was then made acidic and distilled, to ascertain whether volatile acids were produced. The

distillate was made basic and the solvents were evaporated; the residue was treated with *p*-phenylphenacyl bromide to convert any acids present to their *p*-phenylphenacyl esters. The products obtained from this reaction gave GC t_R that corresponded to the t_R of *p*-phenylphenacyl acetate (relative peak area 8.6) and *p*-phenylphenacyl butyrate (relative peak area 1). In addition, traces of p-phenylphenacyl propionate and *p*-phenylphenacyl isobutyrate were observed.

The residue remaining in the distilling flask was diluted with water and acidified. The material was extracted with chloroform. Evaporation of the chloroform layer yielded 13 mg of a yellow material, which was treated with 2,2-dimethoxypropane and two drops of concentrated hydrochloric acid to convert any acidic material present to methyl esters.

Gas chromatography of the product was carried out, but only a large aggregation of unresolved peaks was observed.

The original intent of the pyrolysis of X-5108 was to isolate a major volatile product that had been produced by an earlier pyrolysis.[24]

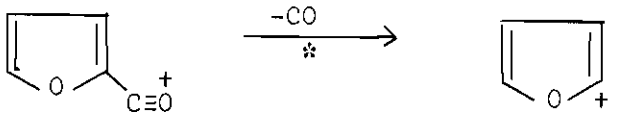
At a column temperature of 100° (6 ft 3% SE-30) a peak of significantly greater area than surrounding peaks appeared at t_R 5.0. A GC-mass spectrum was obtained from this peak. A zinc dust distillation, a sodium hydroxide fusion, and a selenium dehydrogenation had also been carried out, and the product described above had apparently been produced in all three reactions, as observed by GC-MS.[24]

The peaks in the mass spectrum were observed at *m/e* (relative intensity > 1) 138 (9), 123 (3), 121 (2), 119 (2), 110 (3), 109 (2),

107 (2), 96 (8), 95 (100), 93 (2), 91 (3), 81 (3), 71 (3), 69 (2), 68 (4), 67 (46), 66 (3), 65 (8), 63 (3), 58 (4), 57 (3), 55 (6), 53 (4), 52 (5), 51 (6), 44 (6), 43 (17), 42 (5), 41 (36), 40 (4), 39 (22), and 38 (3). Peaks below 38 were not counted.

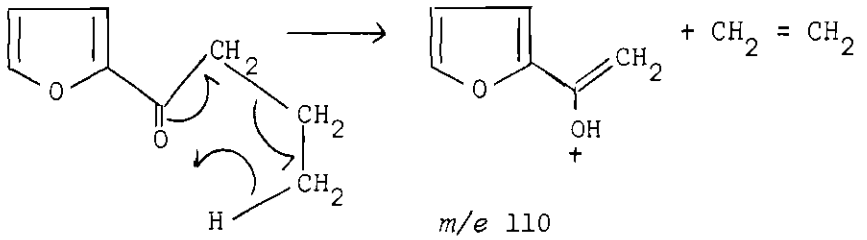
The mass spectrum bears certain resemblances to mass spectra of furans,[25] and the possibility of a furanoid compound is attractive from a genetic point of view, since aromatic compounds are frequently pyrolysis products.

The gas chromatographic results indicate that the compound must be of low molecular weight. Hence, *m/e* 138 seems to be reasonable for the molecular ion. An ion then occurs at *m/e* 123 (M-15). The base peak in the spectrum, *m/e* 95, is M-43. The next abundant ion occurs at *m/e* 67, and is formed from the ion at *m/e* 95 by loss of 28 mass units, as indicated by a metastable peak at *m/e* 47.5. Fairly abundant ions were also observed at *m/e* 43, 41, and 39 (43 = C_3H_7 or C_2H_3O; 41 = C_3H_5; 39 = C_3H_3, thought to be cyclopropylium ion derived from the furan ring). The loss of 28 mass units from the base peak may be loss of either carbon monoxide or ethylene. If loss of carbon monoxide occurs, then the structure of the ions at *m/e* 95 and 67 would be as shown in Equation 3.



m/e 95 *m/e* 67 (3)

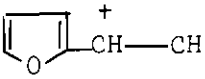
The loss of 43 mass units could then be assigned to loss of propyl or isopropyl groups. The former possibility is excluded, as a spectrum of a known sample of this compound[25] exhibits an abundant McLafferty rearrangement peak at *m/e* 110, which is about 50% of the base peak (*m/e* 95). The rearrangement is shown in Equation 4.

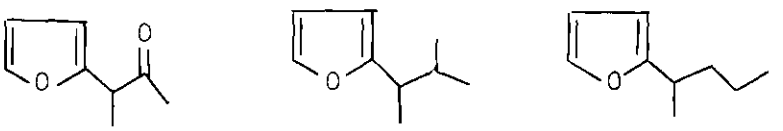


(4)

If a β-furyl compound rather than a α-furyl compound were present, this rearrangement would still occur.

If α-isobutyrylfuran were present, however, no γ-hydrogen would be available for this rearrangement. It is to be noted that in the known spectra of furans, very few abundant ions are present; this is true of the unknown product also.

In addition to the possibility of an isobutyrylfuran, a furan that could lose ethylene from the ion at *m/e* 95 must also be considered. In this case, the ion at *m/e* 95 would be  . There are then three possibilities for the parent molecule; these are I, II, and III.



I II III

Structure I might be eliminated because one would expect a relatively strong abundance of the M-15 ion (*m/e* 123) and the presence of an abundant ion at *m/e* 43. It is not possible to make a clear choice between II or III on the basis of this spectrum.

These interpretations must be regarded as highly speculative in the absence of further data. It should be noted that the ion at *m/e* 67 in the spectra of known furans is not usually as abundant as is indicated in the spectrum of the pyrolysis product. However, since abundances of ions depend upon many variables, this factor was not considered great enough to exclude a furanoid structure.

It was thought that if the pyrolysis were repeated and this compound could be collected, then additional spectral data could be determined and the compound could be identified.

When X-5108 (Na^+) was pyrolyzed, again however, a very large number of compounds were obtained. It must be said that the exact conditions of the previous experiments were not known.

Employing a preparative GC column, it was possible to obtain three peaks only: two of these compounds were collected. The column employed (20 ft 30% SE-30) was so retentive that only very volatile materials would be expected to be eluted.

The nmr spectrum of fraction 2 was identical to that observed for methyl isopropyl ketone, plus impurities (*e.g.*, ether) and therefore was strongly suggestive that methyl isopropyl ketone was present in the pyrolysate. The genesis of this compound is unknown at this time.

Oxidations of X-5108 (H^+) with potassium permanganate were carried out. The first reaction was undertaken in order to observe whether any dicarboxylic acids were produced. The reaction was carried out in aqueous solution; the X-5108 (H^+) was made soluble by the addition of sodium bicarbonate solution. The compound reacted with the permanganate very rapidly; *ca.* 22 moles of permanganate per mole of X-5108 were consumed. This was a surprising finding, as extrapolation of the hydrogenation curve (Figure 1) indicated that five moles of hydrogen per mole of X-5108 were consumed. It must be remembered, however, that these are two different types of reactions, and that X-5108 contains many oxidizable groups.

Ether extracts of the acidified solution yielded material that only exhibited one R_F value that was positive to bromocresol green reagent; this R_F value corresponded to that observed for malonic acid.

Another oxidation was undertaken in order to observe volatile and nonvolatile products. The same procedure was employed, except highly purified X-5108 (H^+) was used for this reaction. The solution resulting from the reaction was filtered, acidified, and distilled; the distillate was made basic and the solvent was evaporated. The residue was treated with *p*-phenylphenacyl bromide in order to convert any acids present to their *p*-phenylphenacyl esters for gas chromatographic analysis.

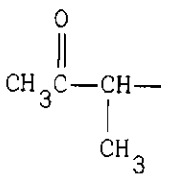
A comparison of the t_R of the products obtained from the previous reaction with those of standard *p*-phenylphenacyl esters of acetic, propionic, isobutyric, and butyric acids indicated that *p*-phenylphenacyl

acetate and *p*-phenylphenacyl butyrate had been produced as a result of the permanganate oxidation.

The residue remaining in the distilling flask was extracted with chloroform to obtain the nonvolatile products. The resulting foul-smelling material was treated with 2,2-dimethoxypropane and hydrochloric acid to convert any acids present to their methyl esters.

Gas chromatography of the resulting material yielded about 30 peaks that were unable to be completely resolved. Several GC-mass spectra were obtained from the more intense peaks, but in most of the spectra it was obvious that a mixture was present. The mass spectrum of the most abundant component of the mixture (as evidenced by GC) appeared to be that of a single compound; this spectrum is given as Plate 3.

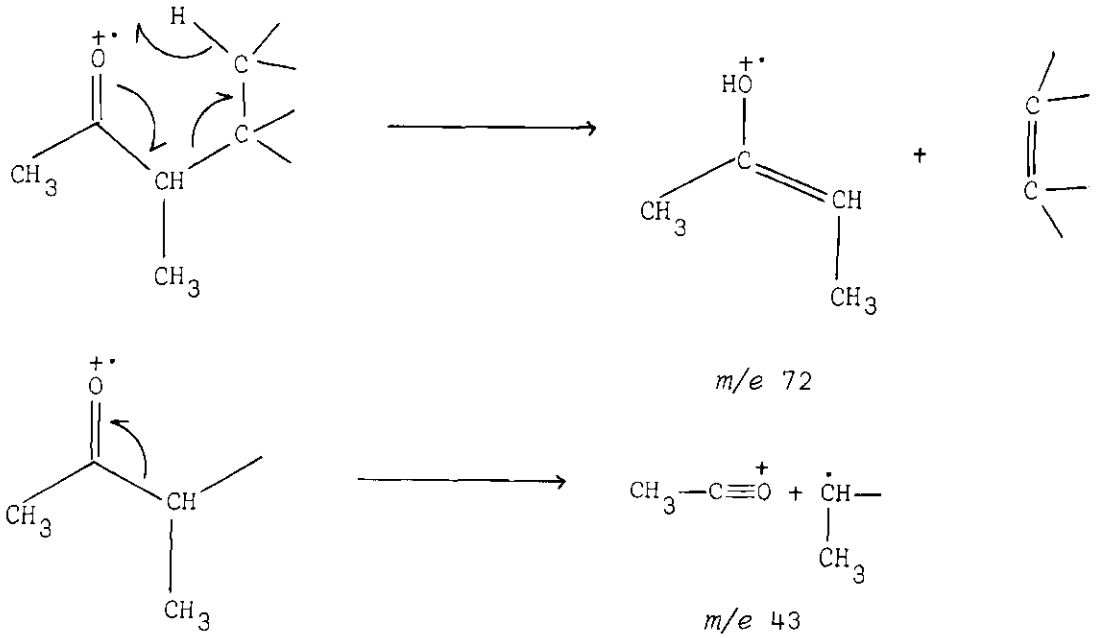
The ion *m/e* 143 is the ion observed at highest mass. If no nitrogen is present, then a molecular ion is not observed. (It does not seem likely that nitrogen is present from consideration of the genesis of the molecule.) Since the loss M-15 is a common occurrence, a molecular ion at *m/e* 158 might be postulated. Losses from this ion to 143 (M-CH_3), 111 (M-CH_3-CH_3OH), and 99 (M-59, $CH_3OC{\equiv}\overset{+}{O}$) are logical and expected for a methyl ester. An ion at *m/e* 59 is present; the presence of an ion at *m/e* 87 and the absence of an ion at *m/e* 74 suggests that the structure



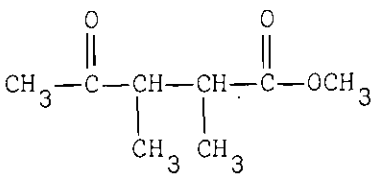
is present (the ion at *m/e* 74, arising from a McLafferty rearrangement, has the structure $[CH_3\overset{OH}{\overset{|}{O}}C{=}CH_2]^{+\cdot}$.

The base peak of the spectrum is *m/e* 72. An abundant ion at *m/e* 43 is also present. In Scheme 1, the genesis of these ions is postulated.

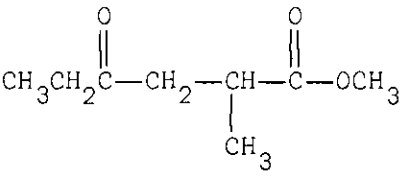
Scheme 1.



The structure of the parent molecule would then be IV.



IV



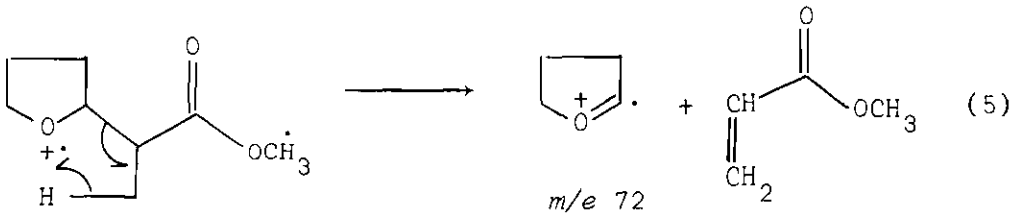
V

However, structure IV would be expected to undergo a McLafferty rearrangement to form an ion at *m/e* 88 involving the ester carbonyl.

Studies[26] have shown that the proclivity for McLafferty rearrangements in a molecule having both ester and carbonyl functions is about equal for each group at 70 eV. There are also no steric factors present that would hinder rearrangement.

Structure V is attractive in that it has no γ-hydrogen available for rearrangement, but the spectrum lacks the expected M-29 ion and an ion at *m/e* 57.

One might also speculate that a ketone is not present, and a tetrahydrofuran structure gives rise to the ion at *m/e* 72 as shown in Equation 5.



(5)

However, in the absence of other spectral data, no conclusions can be made from the available data.

Catalytic hydrogenations of X-5108 were carried out both to observe the amount of hydrogen uptake, and to investigate the reaction products.

It was found that, using 75% acetic acid as the hydrogenation solvent, *ca.* 5 moles of hydrogen per mole of X-5108 (Na^+) was absorbed.

As the uv and nmr spectra indicate a considerable amount of unsaturation in the molecule, these results were not surprising.

The experiments had been carried out using the antibiotic as received.[14] At that time, the presence of lipid impurities was not known and this fact obscured the identification of the true products.

Consequently, these impurities were isolated: a crystalline mixture of stearic and palmitic acids was obtained.

Subsequent hydrogenations using 95% ethanol and in water failed to yield any crystalline or homogeneous material; in either reaction less hydrogen was absorbed per equivalent of X-5108 (Figures 2 and 3) than in the reaction using acetic acid.

A hydrogenation using a methanol-water solvent yielded a white, waxy fraction. Gas chromatography of the material yielded ten peaks; the fraction appeared to be paraffinic in nature from the ir spectrum (2950, 2865, 1464 k) and the nmr spectrum (τ 8.78 and 9.1, relative area 12:1).

When the log t_R of the components was graphed against carbon number, a straight line resulted. These t_R were compared with those of standard *n*-alkanes. Graphical interpolation between compounds of comparable t_R indicated the presence of *n*-$C_{27}H_{56}$, *n*-$C_{28}H_{58}$, *n*-$C_{29}H_{60}$, *n*-$C_{30}H_{62}$, *n*-$C_{31}H_{64}$, *n*-$C_{32}H_{66}$, *n*-$C_{33}H_{68}$, and *n*-$C_{35}H_{72}$.

A GC-mass spectrum was not able to be obtained; therefore a mass spectrum was obtained of the mixture. A typical hydrocarbon ion distribution was observed, with ion clusters spaced 14 mass units apart at *m/e* 43, 57, 71, 85, 99. . . . The ion observed at highest mass was

m/e 621; this suggests that *n*-$C_{46}H_{94}$ might be the compound present of highest molecular weight.

The possibility seemed remote that these high molecular weight *n*-alkanes could have arisen from X-5108. However, to investigate this possibility, a sample of highly purified X-5108 (H^+) was hydrogenated using the same conditions, and the products were subjected to the same isolation procedures. No paraffin-like material was obtained.

It was hoped that catalytic hydrogenation of X-5108 followed by lithium aluminum hydride reduction of the products would result in simple compounds, perhaps alcohols. Accordingly, these reductions were carried out.

The first sequential reduction was accomplished using X-5108 (H^+). After catalytic hydrogenation and lithium aluminum hydride reduction of X-5108 (H^+), a brown oil was obtained. Trituration of this oil with *n*-hexane yielded a colorless oil; gas chromatography demonstrated that this oil was a complex mixture.

The hexane-insoluble material was also subjected to GC. Retention times occurred which suggested a homologous series as evidenced by a graph of log t_R *vs.* carbon number. These t_R were compared with those of *n*-paraffins; a surprising similarity of t_R occurred. One would have expected all *n*-paraffins, if any were present, to have been extracted by *n*-hexane. It is possible that these volatile materials were compounds such as *n*-alcohols whose similarity in t_R was merely coincidental.

In a second reduction, X-5108 (H^+) was reduced as previously described. In this procedure, the filter cake was dissolved in 10%

sulfuric acid and the resulting solution was extracted with 1-butanol: this brown, gummy extract was kept separate from the pale yellow gum obtained from the filtrate.

The brown gum was triturated with *n*-hexane; evaporation of the hexane yielded a colorless oil that demonstrated only one GC peak.

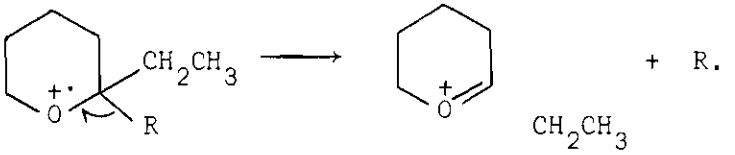
The hexane-insoluble residue demonstrated seven peaks; GC-MS data were obtained for these peaks.

The mass spectrum of the first compound eluted exhibited ions at highest mass *m/e* 193 and 194. It seems unlikely that either of these is the molecular ion by applying the "logical mass differences" test.[27] If these ions are high molecular weight artifacts, then possibly *m/e* 156 is the molecular ion. (Because of the t_R of this peak, this would seem a more likely choice for the molecular ion.) One would then observe a loss of 43 mass units to *m/e* 113, the base peak, and a loss of 57 mass units to *m/e* 99, the second largest peak in the spectrum.

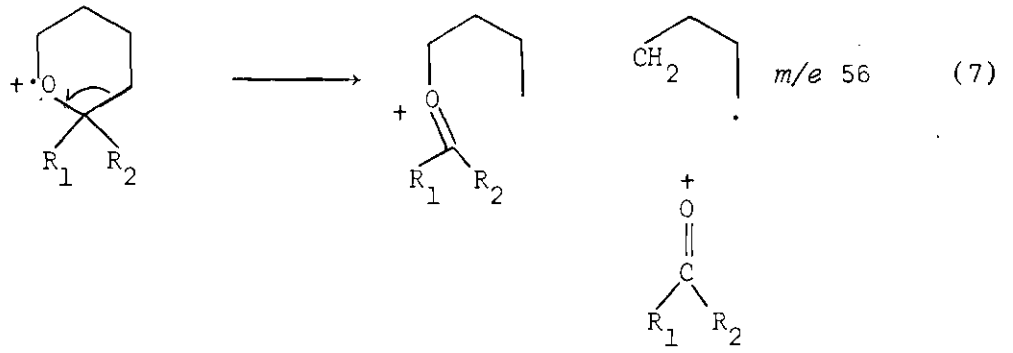
These ions could arise from a saturated branched hydrocarbon, a cyclic ether, or a cyclic alcohol function. There are other isoharic possibilities, but the reductive conditions employed make the stated choices more realistic. The nature of the low mass ion region (absence of the ion series (31, 45, 59, 73, 87, ...) does not seem to indicate the presence of an acyclic alcohol.

If this spectrum were that of a hydrocarbon, one would expect a higher abundance of low mass ions. The ion at *m/e* 56 must be considered; ions having this *m/e* usually are not abundant in the spectra of saturated hydrocarbons.

If one postulates a cyclic ether of the structure VI, then *m/e* 113 could arise by a mechanism as shown in Equation 6. A mechanism such as that shown in Equation 7 could possibly then give rise to *m/e* 56.

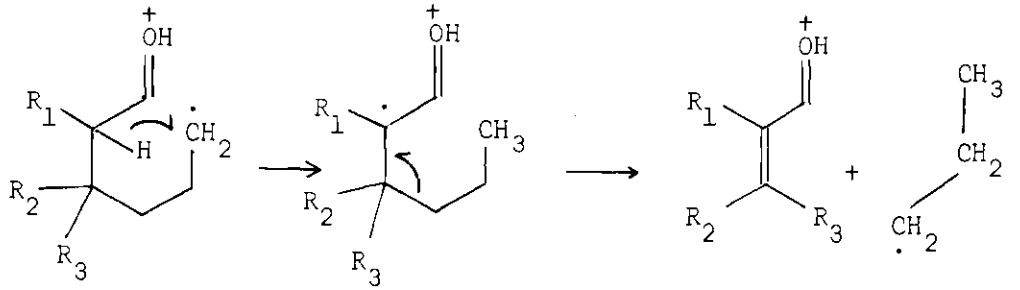


VI *m/e* 113 (6)



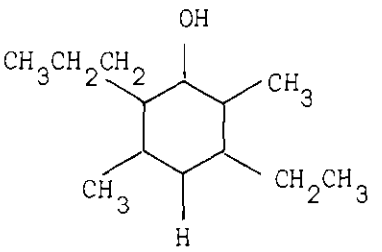
(7)

A discussion of the fragmentation patterns of cyclic alcohols is provided by Budziekiewicz, Djerassi and Williams.[28] If one may generalize the discussion, Equation 8 may be a general fragmentation pattern:



(8)

In this case, if $R_1 + R_2 + R_3 = 45$, an ion of *m/e* 99 would occur, and if $R_1 + R_2 + R_3 = 59$ (e.g., R_1 = Et, R_2 = Me, R_3 = Me) an ion of *m/e* 113 would occur. For example, a possible structure would be structure VII. However, no further interpretation is possible in the absence of further data.



VII

The next GC peak to consider (No. 3) has *m/e* 69 as its base peak; peaks at highest mass occur at *m/e* 225 (9%) and *m/e* 226 (6%). If one considers *m/e* 226 on the molecular ion, a logical loss of M-15 occurs to *m/e* 211 (28%). From the presence of the abundant low mass ion series at *m/e* 41, 55, 69, 83, ..., and the presence of an ion at *m/e* 56, one can suggest that a cyclic molecule is present (excluding unsaturated species). The presence of the abundant odd-electron ion at *m/e* 126 also points to a cyclic molecule. From this mass spectrum it can be said only that possibly a cyclic molecule with one or more hydroxy and/or ether functionalities is present.

Peak No. 4 appears to be similar to the mass spectrum of didehydro X (Plate 14). This is unexpected because of the origin of the products, but it may be that didehydro X is difficult to reduce, since it was found to occur along with compound X (see p. 66).

Peak No. 5 and Peak No. 6 are essentially similar; in both cases the base peak occurs at *m/e* 142 and an abundant peak occurs at *m/e* 114. There are peaks in the spectra that are similar to those of didehydro X; this could be due to a separator tailing effect (very common in the Llewellyn separator[29]) since the GC peaks were well separated. The peaks occurring at highest masses (*m/e* 392, 315) appear to be due to "column bleed" from the OV-17 column. From the appearance of the other peaks in the spectrum, one may only suggest that a cyclic compound with more than one oxygen function is present. Although the two abundant ions at even mass (*m/e* 142, 114) might lead one to suspect the presence of nitrogen, the lack of other even-mass peaks makes this possibility tenuous.

A standard GC run of the pale yellow gum obtained from the filtrate of the reduction indicated that a considerable quantity of material of low molecular weight was present. Consequently, the material was injected into a Porapak Q column, yielding only one peak. Retention times of this peak with those of low molecular weight alcohols were compared; the t_R of *sec*-butyl alcohol was very similar (that of isobutylalcohol was definitely different). Injection of a mixture of the yellow material and *sec*-butyl alcohol yielded only one peak. Injection of a mixture of the yellow material and isobutylalcohol yielded two peaks.

There might be objections to the conclusion that *sec*-butyl alcohol was present, as no further evidence was able to be obtained.

Crystalline material had been found in the products of catalytic hydrogenation of X-5108, and it was desired to obtain optimum methods

for producing and purifying this compound for structural studies. It was found that the material was efficiently produced by hydrogenation under pressure.

Several treatments with carbon, followed by silicic acid and/or silicic acid-carbon chromatography gave a material which could then be further purified by crystallizations from methanol-water.

The white needles obtained from the recrystallizations gave only one tlc spot. Analytical data indicated that the formula was $C_{16}H_{28}O_5$ (mw 300); high resolution mass spectral data confirmed this fact. A saponification equivalent of 432 was obtained, indicating 0.69 groups. It is unusual for a saponification equivalent to be so low; perhaps a group that is difficultly saponifiable is present in the molecule. The active hydrogen analysis gave a value of 1.36% (4.08 active hydrogens). This value is high if one considers that, if one ester or lactone grouping is present in the molecule, then a maximum of three hydroxyl groups could be present. In some cases, however, hydrogens *alpha* to an ester linkage can sometimes be indicated by the Zerewitinoff method, as previously discussed.

The *C*-methyl analysis was 7.84%, indicating at least two groups.

A uv absorption for compound X occurred at λ_{max}^{EtOH} 217 nm, ϵ 1200. The ir (KBr pellet) absorption at 3559 k indicates the presence of hydroxyl groups; the absorption at 1751 k is suggestive of a saturated ester or a saturated lactone, which is consisted with the uv data.

It is unlikely that another type of carbonyl function is present in the molecule, as the compound arose under perhydrogenation conditions.

The absorption at 1639 k is probably due to water, as this absorption does not occur in the ir spectrum of X in chloroform solution. The absorptions in chloroform show a slight hypsochromic shift, with the shift of the carbonyl absorption being the most pronounced, to 1779 k. In this region of the ir spectrum, absorptions due to γ-lactones are observed. Since solution spectra are generally more reliable for correlations,[30] this is very suggestive that a γ-lactone functionality is present in the molecule.

Nmr spectra (100 MHz) of compound X were obtained both in deuterochloroform solution and in pyridine-d_5 solution. Whereas the τ values obtained from the spectrum in deuterochloroform are more suitable for correlations, the spectrum obtained using pyridine-d_5 gave more information. Several of the absorptions in the spectrum of compound X in pyridine-d_5 are shifted downfield from the position observed using deuterochloroform, notably, some of the methyl absorptions. Shifts to lower fields upon going from a nonpolar to a polar solvent have been attributed[31] to the formation of "collision complexes" between solute and polar solvent, where the solute contains groups (e.g. polar groups such as carbonyl or hydrogen-bonding groups) capable of interaction with the solvent. This phenomenon has been utilized in the steroid field to clarify spectra having many absorptions due to methyl groups.[32]

In the deuterochloroform solution, the only obvious multiplets were a quintet at τ 8.22 (2H) and a triplet at τ 7.42 (1H). These two absorptions were apparently coupled to protons at higher field as indicated by the slant of the multiplets.

Two sharp absorptions at τ 9.05 and 9.17 were observed in the region τ 8.96-9.27 (9 H). The spacing between these absorptions was observed to be 12.5 Hz at 100 MHz and 8.0 Hz at 60 MHz, demonstrating that these absorptions were not components of a multiplet. The sharpness of the absorptions indicates that these two absorptions are singlets, ascribable to isolated methyl groups or to a *gem*-dimethyl group.

The region τ 8.50-8.96 (11 H) (deuterochloroform solution), indicating protons attributable to $-CH_2-$ and $-CH\langle$, was complex.

The absorptions that occurred for the pyridine-d_5 solution gave further multiplicities. A 3 H absorption at τ 9.15 was observed to be a distorted triplet by expansion of the region 60-140 Hz. From the expansion, six distinct absorptions, at τ 8.96, 8.89, 8.83, 8.78, 8.76, and 8.69, were also observed, but no unambiguous multiplicity could be inferred from these absorptions.

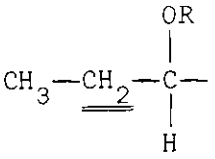
It was observed, however, from the expansion, that the region τ 8.62-8.18 Hz contained 4 H, while the region τ 9.38-8.62 contained 16 H (areas measured by planimetry).

It was noted also that the two apparent singlet methyl groups had been shifted downfield by *ca.* 0.3 Hz which is in the range (0-0.3 Hz) generally observed for solvent shifts.[31]

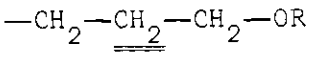
Since one of the methyl groups had not experienced a solvent shift, it may be concluded that the two singlet methyl groups are near a polar center in the molecule where a "collision complex" may be formed, and that one of the methyl groups is remote from this center.

The quintet previously observed occurred at τ 7.90 (2H) in the pyridine-d_5 solution. Expansion gave J = 7.5. A spin-decoupling experiment in which the quintet was irradiated and the absorptions at lower field were observed demonstrated that the quintet apparently was not coupled to lower field protons.

There are then two possibilities for the origin of the quintet: structure VIII and structure IX (R can be H).



VIII



IX

The position of absorption of the quintet (τ 8.22) suggests that it is β to an electronegative group such as hydroxyl or ether oxygen. For example, the β-methylene group in *n*-propyl alcohol absorbs at τ 8.43, [33] whereas the β-methylene group in tetrahydrofuran absorbs at τ 8.15, [34] both in deuterochloroform solution.

A choice may be made between structures VIII and IX by considering the protons α to the oxygen function. A proton in this environment would be expected to absorb at low field; however, there are no two-proton low field absorptions. There are, however, several one-proton low field absorptions. It was stated that apparently no change was

observed in the multiplicities of the lower field protons when the quintet was irradiated; however, no multiplicity at all could be observed for the broad absorption at τ 6.12 (τ 6.55 in deuterochloroform) during the irradiation experiment. Hence, it is possible that this absorption corresponds to the α-proton; other adjacent hydrogens may contribute to the broadness.

The position of this absorption is in the region to be expected for a proton α to an oxygen function; for example, the absorption for the α-hydrogen in 1,3 butanediol is τ 5.97, in 2,3 butanediol, it is τ 6.2, and in 3-methoxybutanol-1, it occurs at τ 6.45 (all in deuterochloroform solution).[35]

These data strongly indicate that part structure VIII is present in the molecule.

There are two doublets that occur at low field in the pyridine-d_5 solution: these absorptions (1 H each) are at τ 5.89 and 5.10. They are shifted downfield from their respective values in deuterochloroform solution (τ 6.35 and 6.10). These doublets appear to become sharper after the solution was allowed to stand with pyridine for several days. Expansion after this time demonstrates symmetrical absorptions, each having J = 4.0. Spin decoupling of either absorption caused the other to collapse to a singlet, demonstrating that the two protons are coupled. The value of J then indicates $-\overset{|}{C}H-\overset{|}{C}H-$. The position of absorption indicates that the protons are deshielded by carbon-oxygen bonds ($-O-\overset{|}{C}H-\overset{|}{C}H-O-$), where the oxygen may be ethereal or hydroxyl.

The absorptions at lowest field occurred at τ 4.75 (1 H) and

τ 1.55 (1 H). The latter absorption was observed 4 hr after sample preparation but disappeared after three days. (No deuterium oxide had been added to the pyridine-d_5 solution). Concomitantly, the absorption at τ 4.75 increased to 2 H. When the solution was concentrated, the absorption at τ 4.75 shifted downfield. This absorption then disappeared when deuterium oxide was added to the solution. These data demonstrate that the absorptions at τ 1.5 and 4.75 are due to hydroxylic protons.

It is unusual for a hydroxyl absorption to occur at such low field (τ 1.55); however, it was found that pyridine shifts the hydroxyl absorption to τ 3.76 from τ 6.51 in deuterochloroform for ethyl lactate. It is thought that the absorption at τ 1.55 in pyridine-d_5 corresponds to the absorption at τ 5.33 in the deuterochloroform solution. It is also unusual that immediate exchange between the hydroxylic protons did not take place. It is possible that the lower field proton is strongly hydrogen bonded: this would affect both the rate of exchange and the position of absorption.

It has been shown[36] that intramolecular hydrogen bonding shifts the position of absorption of hydroxyl groups to lower field. For example, the absorption of the hydroxylic protons of 3-methoxy-4-hydroxyacetophenone[37] is at τ 3.38, while the hydroxyl absorption of 4-isopropylsalicylaldehyde,[38] where hydrogen bonding is possible, occurs at τ -1.00.

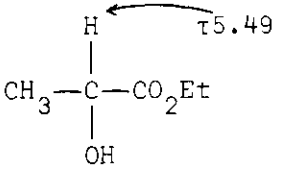
The nmr spectrum of compound X in pyridine-d_5 demonstrated other changes after the addition of deuterium oxide. The triplet at τ 7.15 (J = 7.0) that had been observed at τ 7.42 in deuterochloroform was

present 1 hr after the addition of deuterium oxide to the pyridine-d_5 solution, but disappeared one day later. The position of absorption was in the region for protons α to carbonyl groups; therefore model compounds were studied in order to determine in what types of compounds having protons in this environment exchange would occur.

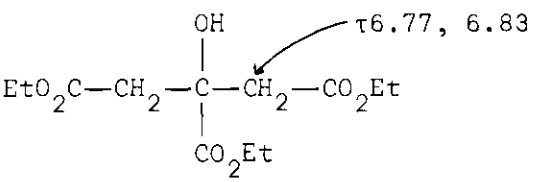
The spectra of ethylacetoacetate and 4-valerolactone were determined, deuterium oxide was added, and the spectra were determined four days later (see Table 7). It was observed that the methylene protons between the two carbonyls in ethyl acetoacetate had exchanged almost completely. A small amount of exchange also occurred at the methyl group α to the carbonyl, and apparently, some exchange occurred at the methylene group α to the ester oxygen.

The spectrum of 4-valerolactone indicated only a small amount of exchange for the protons α to the carbonyl group.

It was thought that a hydroxyl group located α or β to the carbonyl function might promote exchange. The model compounds ethyl lactate (X) and triethyl citrate (XI) were then studied before and after the addition of deuterium oxide (pyridine solution). The τ values given below are those determined using pyridine.

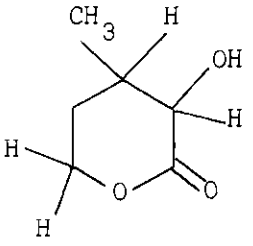


X

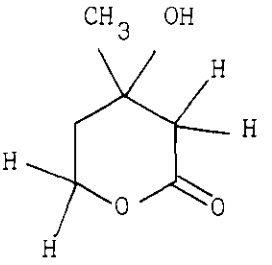


XI

No exchange was found to take place ten days after adding deuterium oxide. An α-hydroxy ester could then be ruled out on the basis of the position of absorption; in structure XII the absorption of the α-proton occurs at τ 6.13.[39] In structure XIII, however, the α-proton absorptions occur at τ 7.49 and τ 7.32 (deuterochloroform solution), which is in good agreement with the value of τ 7.42 (deuterochloroform solution) observed for the triplet in compound X.



XII

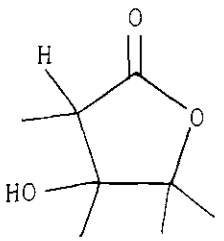


XIII

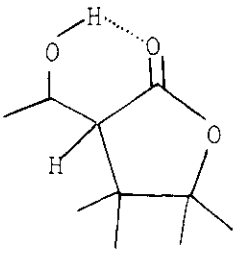
Conclusions to be drawn from the above data must be partly speculative. First, although it seems that an enolic β-keto lactone is the most likely choice for exchange, the ir and uv spectra rule out the presence of this functionality in the molecule. Furthermore, since the molecule arose under perhydrogenation conditions, one would expect no keto or enol groups to remain. Any other structures involving unsaturated groups may also be eliminated on this basis.

From the position of absorption, then, a tentative conclusion might be made that the part structure present is XIV or XV. There might

be factors present such as hydrogen bonding or stereochemistry that could contribute to the exchange.



XIV



XV

Another occurrence which might be called anomalous was observed after the addition of deuterium oxide to the pyridine-d_5 solutions. The two doublets previously observed (100 MHz) at τ 5.89 and 5.10 in the pyridine-d_5 solution were then observed as triplets at similar absorption positions.

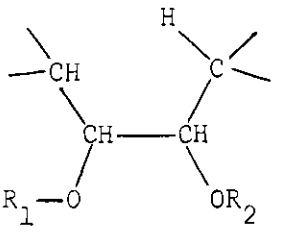
When a spectrum was obtained using the 60 MHz instrument, two four-line spectra were obtained: for τ 5.12, expansion gave J = 4.0 and 2.6, and for τ 5.83, expansion gave J = 4.0 and 1.8.

Expansion was attempted using the 100 MHz instrument, but only two triplets were observed. This must be attributed to the poor resolution of the 100 MHz instrument at that time.

Spin decoupling experiments were nevertheless carried out; when each triplet was irradiated, the other collapsed to a doublet. Expansion of the decoupling wherein the absorption at τ 5.10 was irradiated indicated that the absorption at τ 5.89 collapsed to a doublet with J = 1.2.

From these data one may tentatively conclude that stereochemical factors give rise to the difference in multiplicity observed in pyridine-d_5 and deuterium oxide-pyridine-d_5 solutions. The Karplus relationship[41] of coupling constant to dihedral angle could be a factor here: the deuterium oxide may change the conformation of the molecule by interacting with it and thus change the dihedral angle between the protons.*

The implication of this conclusion then is that the low field protons are present in a stereospecific relationship such as that in a ring structure. The part structure XVI may then be suggested.



XVI

The mass spectrum of compound X is given as Plate 5; the high resolution mass spectrum is given as Table 6. A mass spectrum was obtained from deuterated compound X (Plate 7). From an examination of these spectra and consideration of the functional complexity of compound X, it is apparent that not many *a priori* structural assignments can be made; nevertheless some information may be gained.

The molecular ion is observed at *m/e* 300; high resolution

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*Lemieux[42] found that the conformation of methyl 2-deoxy-α-riboside in chloroform (where internal hydrogen bonding may occur) is different from the conformation in water; this difference was observed by nmr.

measurements confirm the formula as $C_{16}H_{28}O_5$ (observed, 300.1931; calcd, 300.1937). An expansion of the spectrum of deuterated compound X (obtained from a solution of compound X in pyridine-d_5-D_2O by evaporation of the solvents) in the molecular ion region indicated that three deuterium atoms were present.

An ion was observed for compound X at *m/e* 299; its abundance was 40% of the abundance of *m/e* 300. It was desired to eliminate this fragmentation so as to more accurately determine the number of deuterium atoms present in the molecular ion of deuterated compound X; therefore the molecular ion region of compound X was scanned at low electron energies.[43] Even at 15 eV this fragmentation (M-1) was not suppressed. One may suggest, then, that a structure is present that loses hydrogen very readily upon electron impact--for example, a cyclic or substituted ether function.[44]

An ion at *m/e* 282 ($C_{16}H_{26}O_4$) indicates loss of a molecule of water; this transition is confirmed by a metastable peak at *m/e* 265 (calcd 265.1). Loss of water would be expected from the alcohol functions present in the molecule.

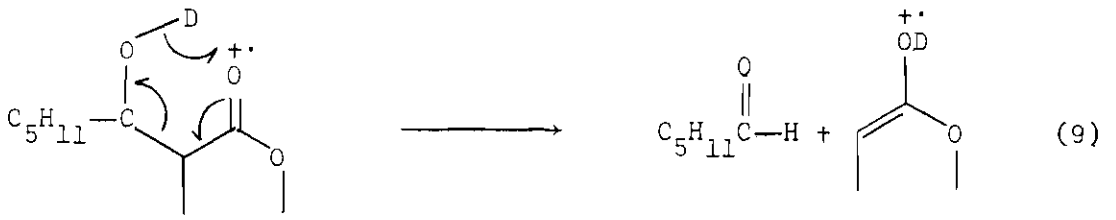
An ion is observed at *m/e* 231 ($C_{12}H_{23}O_4$), which corresponds to loss of C_4H_5O from the parent molecule. This is confirmed by a metastable peak at *m/e* 178 (calcd 177.9). It is possible that this ion then loses a molecule of water to form the ion at *m/e* 213 ($C_{12}H_{21}O_3$). The ion at *m/e* 231 contains only two deuterium atoms in X-d_3; this indicates that one of the hydroxyl groups or that the exchangeable C-H group is contained in the fragment C_4H_5O. Since it is obvious that this ion

cannot result from simple cleavage, no further information can be elucidated from this fragmentation at this time.

An unusual feature of the mass spectrum of compound X is the large number of abundant odd-electron ions. These ions must result from rearrangements of the McLafferty type and/or from ring cleavages.

The first of these ions occurs at *m/e* 228 ($C_{12}H_{20}O_4$), and corresponds to a loss of C_4H_8O from M^+. It is not clear whether this ion gains two or three deuterium atoms because of the overlap with *m/e* 231. It is unlikely that this ion could arise from *m/e* 282 (loss of C_4H_6) or *m/e* 231 (loss of 3H). Loss of 3 H is rarely encountered.[45]

The next abundant ion occurs at *m/e* 200 ($C_{10}H_{16}O_4$), a loss of 100 mass units ($C_6H_{12}O$) from the parent molecule. This ion contains three deuterium atoms in X-d_3; it would seem to follow that the ether function is present in the fragment cleaned. However, the possibility of rearrangements of the labile deuterium atoms must be considered when making conclusions of this type. A McLafferty type rearrangement[46] as shown in Equation 9 would result in elimination of $C_6H_{12}O$ and retention of three deuterium atoms.



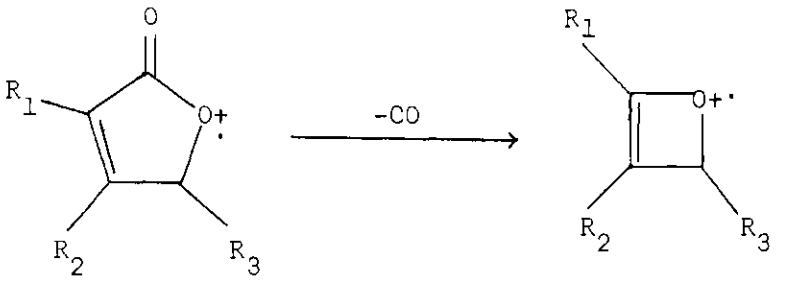
(9)

The next abundant ion occurs at *m/e* 182 ($C_{10}H_{14}O_3$) and retains only one deuterium atom in X-d_3. A loss of $C_6H_{14}O_2$ may be loss of $C_6H_{12}O + H_2O$, and the ion could arise from the transition 300 → 182, 282 → 182, or 200 → 182. However, no metastables are present to verify any of these transitions.

It must be noted that the ion at *m/e* 182 (183 in X-d_3) contains *two* deuterium atoms less than *m/e* 200 (203 in X-d_3); therefore, the ion at *m/e* 182 would have to arise from loss of D_2O rather than HOD. This would then require a specific arrangement of $-\overset{|}{\underset{|}{C}}-D$ and $-\overset{|}{C}-OD$ in the molecule, because in acyclic alcohols, it has been shown[47] that elimination of water takes place by a specific 1,4-dehydration, whereas in cyclic alcohols,[48] elimination takes place by either a 1,4- or a 1,3-mechanism.

From the ion at *m/e* 182 ($C_{10}H_{14}O_3$), a loss of carbon monoxide then occurs to form the ion at *m/e* 154 ($C_9H_{14}O_2$). This transition is verified by a metastable peak at *m/e* 130.5 (calcd. 130.3). The peak at *m/e* 154 also contains only one deuterium atom in X-d_3 (*m/e* 155).

The carbon monoxide ejected probably arises from the lactone ring of the molecule. Saturated γ-lactones do not eject carbon monoxide to any great extent,[49] but the ejection is a facile process in α,β-unsaturated lactones.[50] Hence, one may suggest that the process involving ions at *m/e* 182 and 154 is of the type represented by Equation 10.



(10)

The next abundant ion is at *m/e* 128 ($C_6H_8O_3$). It contains two deuterium atoms in X-d_3, thus it does not arise from loss of hydrocarbon fragments from the ions *m/e* 182 and *m/e* 154. It is possible that the ion arises by loss of C_4H_8O from *m/e* 200, but a metastable peak for this loss does not occur; a metastable peak is observed at *m/e* 80, but the calculated value of 81.9 seems too high.

The ion at *m/e* 128 can then lose carbon monoxide to form *m/e* 100 ($C_5H_8O_2$). A metastable peak at *m/e* 78.2 (calcd 78.1) confirms this transition. Again we may assume that the lactone carbonyl is involved. it should also be observed that a contribution to *m/e* 100 is also made by $C_4H_4O_3$.

The next abundant ion is at *m/e* 85. This ion has as its main contributor C_5H_9O. Contributions from C_6H_{13} and $C_4H_5O_2$ are also observed. The ion contains *no* deuterium when compound X is deuterated. The temptation is strong to assign a structure such as

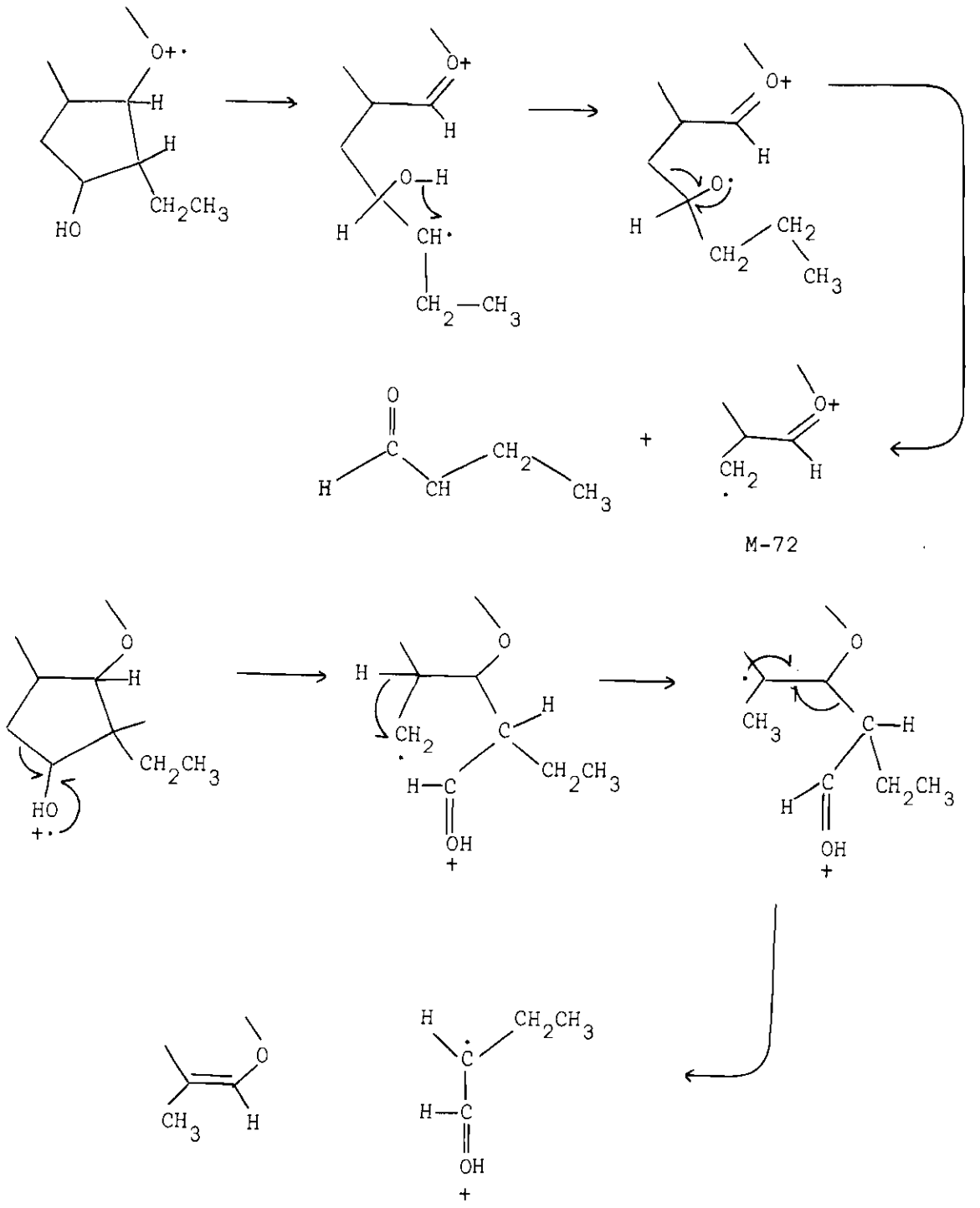
to this ion, but the nmr data rule out the latter possibility as being present in the molecule (no $-CH_2-O-$ absorption in the nmr spectrum). The former is possible, but it is more likely that the ion arises from a ring cleavage involving either the lactone oxygen or the ether oxygen.

The base peak in the spectrum is the ion *m/e* 72 (C_4H_8O), which contains one deuterium atom in X-d_3. There are two transitions in which the fragment C_4H_8O may be lost: 300 → 228 and 200 → 128. When considering the fragmentations of a compound containing many heteroatoms capable of carrying the charge and thus directing fragmentation, it is possible that retention of charge may be held by either of two oxygen-containing fragments.

Hence, it is possible that *m/e* 72 arises from *m/e* 200 by elimination of $C_6H_8O_3$. A metastable ion would be expected at *m/e* 25.9 (calcd); one is observed at this position. It is to be noted that *m/e* 72 cannot arise from *m/e* 100 by loss of carbon monoxide, as *m/e* contains two deuterium atoms in X-d_3 and *m/e* 72 contains only one deuterium atom X-d_3.

Scheme 2 gives mechanisms for both loss of a neutral fragment of mass 72 and formation of the ion with *m/e* 72.

Scheme 2



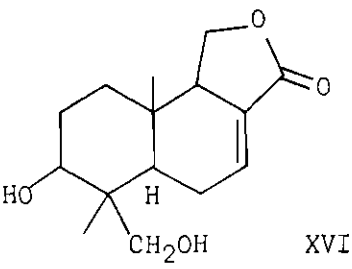
A compound that was present as an impurity in compound X, and was produced from compound X by treatment with concentrated hydrochloric acid, was called didehydro X.

The formula of the compound as a didehydro product of compound X was confirmed as $C_{16}H_{24}O_3$ by high-resolution mass spectrometry (observed, 264.1726; calcd, 264.1725). The didehydro compound was found to be a ubiquitous product in reactions of compound X. A monodehydro product was also observed.

It was not possible to obtain didehydro X in crystalline form, but the product having a single spot by tlc and a single peak by GC had the following spectral data.

The ir spectrum (Plate 13) below 1600 k had essentially the same features as compound X except for the appearance of a strong absorption at 1673 k, attributable to a double bond. The carbonyl absorption, centered at 1760 k in chloroform solution, was broadened slightly; the bathochromic shift suggests that an α,β-unsaturated γ-lactone functionality has been formed. An absorption due to hydroxyl was also observed. This was unexpected; the absorption could be due to a small amount of water present.

The uv absorption of didehydro X occurred at 236 nm, ε 9000. A calculation for either an *endo* or *exo* double bond for an α,β,β-substituted γ-lactone ring system gives a value of 232 nm. For comparison, iresin (XVII)[51] has a λ_{max} of 224 nm, log ε 4.16; the calculated value for an α,β- substituted γ-lactone is 222 nm.



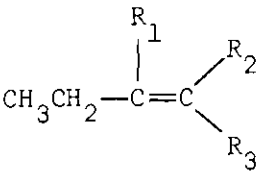
XVII

The above data then indicate that an α,β-unsaturated γ-lactone system is present in dehydro X.

A 100 MHz nmr spectrum of didehydro X in deuterochloroform (Plate 15) was obtained. The region of the spectrum 8.4-9.2 τ of didehydro X was very much like that of compound X, except for the downfield shifts of the two sharp absorptions at τ 9.05, 9.17 in X to τ 8.91, 9.01 in didehydro X. The shift indicates that the methyl groups giving rise to these absorptions are deshielded by the presence of an unsaturated system in the didehydro compound. The position of absorption indicates that these groups are no greater than two carbon atoms removed from the site of unsaturation.

The quintet present in the spectrum of compound X is no longer recognizable in the didehydro X spectrum.

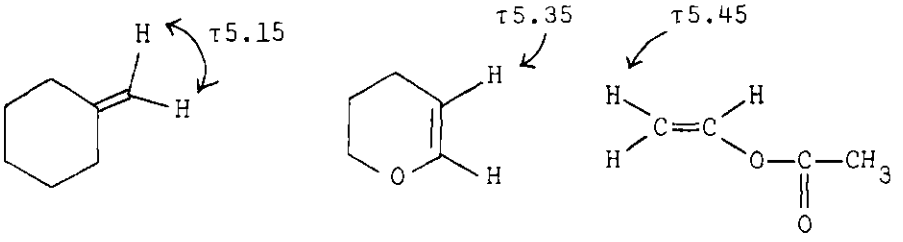
Perhaps the most salient feature of the nmr spectrum of didehydro X is the quartet at τ 7.80. The appearance of this quartet indicates that it is coupled to an upfield absorption, and the position of absorption indicates that the group is α to an unsaturated linkage. Although the integral could not be determined with accuracy, the appearance of the multiplet suggests 2 H. The part structure XVIII is then possible (R_1 not H).



XVII

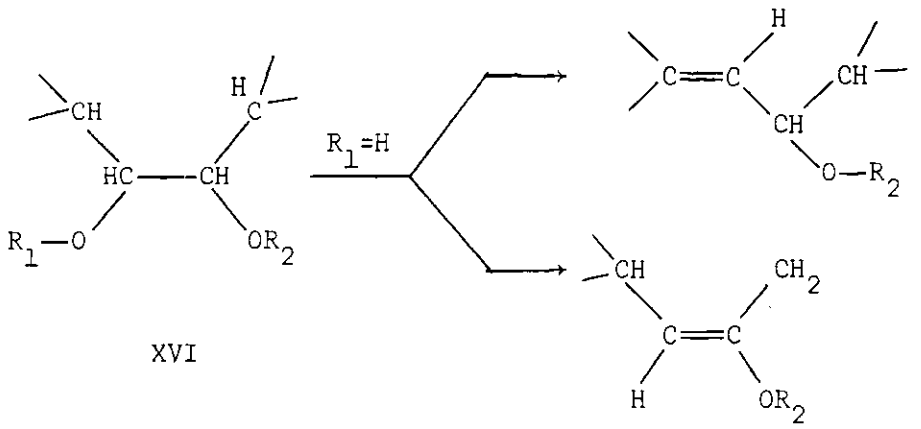
The lack of clarity in the region τ 6.1-7.5 suggests that the didehydro X used for the nmr sample was slightly impure.

There are three low-field, presumably one-proton, doublets in the nmr spectrum of didehydro X, each with J=4.0, at τ 5.10, 5.57, and 5.99. No absorptions are present at lower field. The presence of olefinic protons then requires specific structural features: only protons of exomethylene groups, terminal methylene groups, and protons β to ether functions absorb in the region τ 5-6. For example, the olefinic nmr absorptions of compounds XIX, XX, and XXI are as indicated.



XIX XX XXI

It is also possible that no olefinic protons are present; however, the part structure XVI requires at least one olefinic proton to be formed upon dehydration, unless both R groups are alkyl.



XVI (11a) (11b)

Equation 11b would allow a product having a proton which could absorb in the region τ 5-6. One may conclude that the absorptions are due to either the above-mentioned structures or to structures in which the protons are deshielded by an adjacent double bond (—CH—C═C).

A low-resolution mass spectrum (Plate 14) and a high-resolution mass spectrum (Table 11) were obtained of didehydro X.

The molecular ion (*m/e* 264) has the formula $C_{16}H_{24}O_3$, corresponding to a molecule with two molecules of water less than compound X. The next major ion occurs at *m/e* 249 ($C_{15}H_{21}O_3$), which is loss of methyl from the molecular ion. (This loss does not occur in compound X.) A molecule of carbon dioxide is lost from the molecular ion to give the ion at *m/e* 236 ($C_{15}H_{24}O_2$).

The ion at *m/e* 221 ($C_{14}H_{21}O_2$) corresponds to loss of C_2H_3O from *m/e* 264 ($C_{16}H_{24}O_3$). This could be loss of (CH_3+CO), or it could be loss of CO from *m/e* 249, or possibly loss of $-CH_3$ from *m/e* 236. Since, however, no metastable data are available for this compound, these possible transitions cannot be clarified.

The ion at *m/e* 207 is made up of two species of approximately equal intensity: $C_{13}H_{19}O_2$ and $C_{12}H_{15}O_3$. The former ion corresponds to a loss of C_3H_5O (possibly C_2H_5+CO) from the molecular ion, or loss of C_2H_5 from *m/e* 236. The latter ion represents a loss of C_4H_9 from the parent molecule: if this is a real transition, it indicates the presence of a butyl group in the molecule.

The ion at *m/e* 193 has the formula $C_{11}H_{13}O_3$, indicating loss of C_5H_{11} from the molecular ion; the ion at *m/e* 179 ($C_{10}H_{11}O_3$) indicates the loss of C_6H_{13}. Loss of C_6H_{13} does not necessarily indicate the presence of a hexyl group in the molecule, since the ion could have been formed by loss of ethylene from *m/e* 207 ($C_{12}H_{15}O_3$).

One of the most abundant ions in the spectrum is *m/e* 167. This ion is made up to the greatest extent by $C_{11}H_{19}O$, which corresponds to loss of $C_5H_5O_2$ from the parent molecule. It is possible that this fragment lost is related to the lactone ring. It may also be noted that this loss corresponds to loss of (CO+C_4H_5O) from the molecular ion: the loss of C_4H_5O from M+ is observed in the spectrum of compound X.

The peak at *m/e* 167 is also made up of $C_9H_{11}O_3$, which corresponds to loss of C_7H_{13} from M^+. The peak at *m/e* 165 has the formula $C_9H_9O_3$, which might be ascribed to loss of H_2 from *m/e* 167 rather than loss of C_7H_{15} from M^+. An ion at *m/e* 153 ($C_8H_9O_3$) possibly arises from loss of C_8H_{15} from M^+.

At this point it might be commented that the presence of a lengthy alkyl chain, saturated or unsaturated, in the molecule of dide-hydro X seems incompatible with the data obtained for compound X.

Thus the possibility of a skeletal rearrangement upon going from compound X to didehydro X must be kept in mind before concluding any structural features of compound X from the data obtained for didehydro X.

The ion at *m/e* 151, then, corresponds to loss of $C_7H_{13}O$ (possibly C_6H_{13} + CO) from M^+, and *m/e* 137 could arise from loss of (C_7H_{15} + CO) from M^+.

A hydrocarbon ion (C_9H_{16}) occurs at *m/e* 124; this might arise from loss of (CH_3 + CO) from *m/e* 167 ($C_{11}H_{19}O$).

At *m/e* 109, an abundant ion has the formula C_8H_{13} and differs from the ion at *m/e* 124 by —CH_3. It might be pointed out, however, that the ion at *m/e* 97 is composed mainly of $C_5H_5O_2$; this fragment is lost from $M^{+\cdot}$ to form the abundant ion at *m/e* 167.

The lower mass ion series is typical of unsaturated, cyclic, or cyclic oxygen-containing compounds.[52]

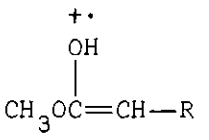
Because of the functional complexity of the compound X molecule, it was thought that structural elucidation by X-ray crystallographic analysis might be a preferred route. Therefore, an attempt was made to introduce a bromine atom into the molecule by preparation of a bromoacetyl derivative of compound X using bromoacetyl bromide.

It was found that this reaction did not succeed: examination of the yellow ether extract of the black, tarry product revealed many compounds present; GC-MS demonstrated that no volatile bromo compounds had been formed; however, the mass spectrum of one of the peaks appeared to be that of didehydro X (Plate 14).

It was thought that basic hydrolysis of compound X might yield information about the carboxyl function in the molecule. Accordingly, the hydrolysis was carried out using 20 per cent sodium hydroxide at 70°.

The aqueous solution resulting was made acidic and extracted with ether. The residue left after evaporation of the ether was treated with 2,2-dimethoxypropane and hydrochloric acid to convert any carboxylic acids present to their methyl esters. The reaction mixture was allowed to stand overnight and was then injected directly into the gas chromatograph. There were several components present; a GC-MS study was made. Table 9 gives a summary of the GC-MS data obtained.

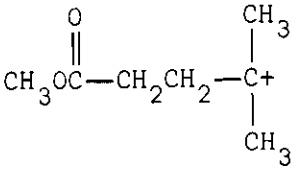
The mass spectrum of the first GC peak eluted does not appear to be that of a methyl ester. There is no ion present at *m/e* 59 ($CH_3OC\equiv\overset{+}{O}$), and no member of the series $[CH_3\overset{O}{\overset{\|}{C}}-(CH_2)_n]^+$ (73,87,101,115,129,...) is present. In addition no McLafferty rearrangement ion



characteristic of methyl esters is observed.

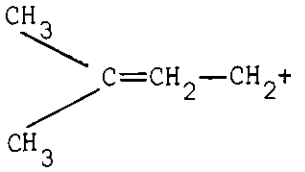
It is not possible to observe the molecular ion; none of the ions present at highest mass seem to be a good choice. The base peak is observed at *m/e* 69; this peak is a member of the series (41,55,69,83, 97,111,125,139) which is present. The presence of this series suggests a cyclic or unsaturated alkyl system. These ions may also be formed from cyclic ketones.[52]

The GC peak No. 2 does not exhibit a molecular ion. However, an abundant ion is found at *m/e* 59, suggesting the presence of a methyl ester. The base peak is found at *m/e* 129, which corresponds to the formula $[C_5H_{10}CO_2CH_3]^+$. Since there are no abundant ions of lower mass in this series, it may be concluded that either the molecule has cleaved at a quaternary branching point, or that there is a C_4 side chain attached to the carbon α to the carbonyl.[53] However, from the six possible structures that may be drawn for the ion, only structure XXII has features that are consistent with the nmr spectrum of compound X.



m/e 129

XXII

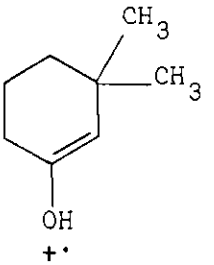


m/e 69

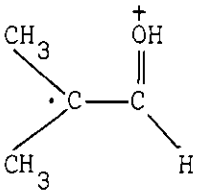
XXIII

There is also an abundant ion at *m/e* 69 in the spectrum; this ion occurs in the spectrum of methyl 4,4-dimethylheptane-1,4-dioate[53] and has structure XXIII. Abundant odd-electron ions are present in the spectrum of peak No. 2 at *m/e* 56, 70, 72, and 126; these ions are not present in the spectrum of methyl 4,4-dimethylheptane-1,4-dioate.

Ions at *m/e* 56 and 70 are commonly encountered as ions of the formula C_nH_{2n} and usually arise from cyclic or unsaturated compounds. Ions having *m/e* 126 are sometimes encountered in the spectra of long-chain dimethyl esters and may have structures such as XXIV.[54]



XXIV



XXV

An ion at *m/e* 72, having the formula C_4H_8O, is observed in the spectrum of compound X; perhaps a possible structure is XXV.

Hydroxy esters, of course, would be the expected products of this reaction. However, in the absence of a well-defined molecular ion, no definite conclusions can be made concerning the structure of this product.

The third GC peak appears to be another unusual compound. The base peak is observed at *m/e* 185, and the peak at highest mass is observed at *m/e* 250. A loss to *m/e* 185 would be M-65: this does not seem to be a logical loss for a methyl ester or a substituted (hydroxy, oxo, etc.) methyl ester.

The ion at *m/e* 185 could correspond to $[C_9H_{18}CO_2CH_3]^+$; possibly $[O{\equiv}C{-}C_7H_{14}{-}CO_2CH_3]^+$. There are no McLafferty saturated ester rearrangement peaks in the spectrum; this is not characteristic of keto

esters. An abundant odd-electron ion appears at *m/e* 154 to further complicate the picture. The compound cannot be identified on the basis of this spectrum.

The fourth GC peak exhibits abundant ions at *m/e* 69, 97, 124, 125, 140, 157, and 167. The ion at *m/e* 157 is isobaric with the "characteristic methyl ester" peak of formula $C_nH_{2n}CO_2CH_3$ (n=7) or the formula where $-CH_2-CH_2-$ is replaced by carbonyl. Ions characteristic of didehydro X are observed in the upper region of the spectrum; this GC peak appears to be a mixture.

The fifth peak exhibits ions characteristic of didehydro X.

The sixth GC peak appears also to be contaminated with didehydro X, but a base peak is observed at *m/e* 199, and an abundant ion is observed at *m/e* 129, both analogous of $CH_3O\overset{O}{\overset{\|}{C}}-C_nH_{2n}$; however, *no m/e* 59 is observed.

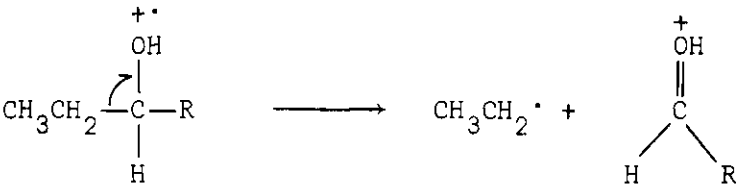
The seventh peak exhibits peaks characteristic of a compound encountered previously as a product of sequential reductions (see Table 5). The last peak exhibits ions characteristic of monodehydro X.* (See Plate 16.) It is noteworthy to compare the differences in fragmentation patterns between mono- and didehydro X.

The molecular ion of monodehydro X is observed at *m/e* 282. A loss of H_2O (M-18) to *m/e* 264 is then observed; the resultant didehydro X then fragments as previously observed. The molecular ion (*m/e* 282)

---

*Monodehydro X has been observed frequently by GC-MS; it was isolated as a semicrystalline material from an attempted preparation of didehydro X by reaction of compound X with formic acid. The compound was contaminated with an amount of didehydro X from which it could not be separated.

loses 29 mass units (C_2H_5) to give *m/e* 253. Loss of ethyl is not observed in the spectrum of X or of didehydro X (unless ethyl is lost from an ($M^{\dagger}$—CO) species). This could indicate that in monodehydro X the presumed $CH_3—CH_2—\underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}}—$ group is still present to cleave as indicated in Equation 12.[55]



(12)

It is to be noted that an abundant *m/e* 59 ion is not present; this would correspond to $CH_3CH_2\overset{\overset{H}{|}}{C}=\overset{+}{O}H$. The latter transition conforms to the rule of the largest group being lost in alcohol cleavages,[56] but in complex molecules where many functions may direct cleavages, such rules do not always apply.

The next ions purely characteristic of monodehydro X occur at *m/e* 157 and *m/e* 155. These correspond to losses of 125 and 127 mass units; it might be noted that the ions at *m/e* 125 and 127 are abundant also.

Abundant odd-electron ions occur at *m/e* 128 and 140. The ion at *m/e* 128 occurs in the mass spectrum of X; however, *m/e* 100, to which *m/e* 128 decays by loss of carbon monoxide, is not present in the spectrum of monodehydro X.

The base peak is observed at *m/e* 97. This ion is observed as a very strong peak of didehydro X. Other characteristic low mass ions of didehydro X are also present.

The peak at *m/e* 85, present in the spectrum of X but not of didehydro X, is present in the spectrum of monodehydro X.

This indicates that the part of the molecule giving rise to the ion at *m/e* 85 is near to a hydroxyl group that influences its formation (however, the fact that *m/e* 85 appears virtually intact in the spectrum of X-d_3 precludes its containing a hydroxyl group.)

A nitric acid oxidation of compound X was carried out with the hope that simple carboxylic acids, which could be identified by GC-MS of a suitable volatilizing derivative, would be produced.

The usual oxidation procedure was employed. After the reaction had taken place, the solution was steam distilled to remove volatile acids. The distillate was concentrated and treated with *p*-phenylphenacyl bromide to convert any acids present to their *p*-phenylphenacyl esters. The resulting material was found to contain *p*-phenylphenacyl acetate and propionate by comparison of GC retention times with those of standard *p*-phenylphenacyl esters.

A second oxidation was accomplished; the *p*H of the solution was adjusted to 1.5 and the solution was extracted with chloroform. The residue from the chloroform extract was dissolved in methanol and treated with 2,2-dimethoxypropane and hydrochloric acid to convert any acids present to their methyl esters.

The resulting material was subjected to GC-MS. Some 18 GC peaks were observed; only eight peaks were of the abundance necessary to obtain mass spectra. Of these peaks, one peak constituted *ca.* 80% of the total peak area.

The mass spectra obtained are given as Table 10. It was expected that simple dicarboxylic acid methyl esters would be obtained; an examination of the spectra reveal no compounds that correlate with known compounds.[53,57]

The discussion is, therefore, concerned chiefly with the mass spectrum of the major product; the minor products are discussed only as correlations with other spectra.

It should be noted that nitric acid is a vigorous oxidizing agent, and that there are many possible unexpected reaction pathways.

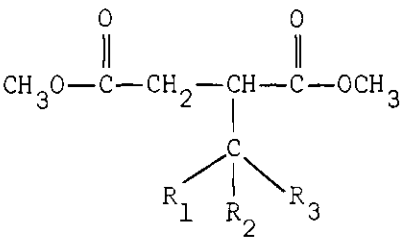
The major product is GC peak No. 12. The ion observed at highest mass is at *m/e* 255 (rel intensity 36). Since this ion cannot be the molecular ion, a reasonable choice is *m/e* 286. An abundant ion at *m/e* 59 indicates that one or more carboxymethyl groups are probably present; therefore if *m/e* 286 was the molecular ion, 31 mass units ($-OCH_3$) could be lost to form *m/e* 255, 73 mass units ($-CH_2CO_2CH_3$) could be lost to form *m/e* 213, and 91 mass units ($-CO_2CH_3$,$-OCH_3$,$-H$)[53] could be lost to form *m/e* 195. A loss of 101 mass units ($-C_3H_6CO_2CH_3$) could then give *m/e* 185. It is then possible that the formula for this last ion is $[C_9H_{18}CO_2CH_3]^+$. However, this would require that the formula for the parent molecule be $C_{12}H_{26}(CO_2CH_3)_2$, indicating a 14-carbon acid resulting from compound X! Clearly this cannot be the case, considering the

five oxygen atoms present in X ($C_{16}H_{28}O_5$).

Another possible formula for the ion would be $[C_8H_{16}OCO_2CH_3]^+$; this formula would require one ring (double bonds cannot be present and therefore the formula for the parent molecule would be $C_{11}H_{22}O-(CO_2CH_3)_2$. The possibility that a tricarboxylic acid is present is eliminated by a consideration of the isotope ratios for the peak at *m/e* 255. A peak measurement yielded ratios of 100, 16.7, and 2.8 for M, M + 1, and M + 2, respectively. The formulas $C_{15}H_{27}O_3$ for this ion would have isotope ratios of 100, 16.6, and 1.9; the formula $C_{14}H_{23}O_4$ would have ratios of 100, 15.8, 1.9; the formula $C_{13}H_{19}O_5$ would have ratios 100, 14.5, 2.0. These are all possible, but a tricarboxylic acid would require the formula $C_{11}H_{17}O_7$ and would have the ratios of 100, 12.4 and 2.17.

An examination of the lower mass ion region reveals no further ions of the formula $[C_nH_{2n}CO_2CH_3]^+$. It is observed, however, that the series *m/e* 41, 55, 69, 83, 97, 111, 125, 139, 153, 167 and 195 is present, with the absence of the number *m/e* 181. This could indicate a point of branching.

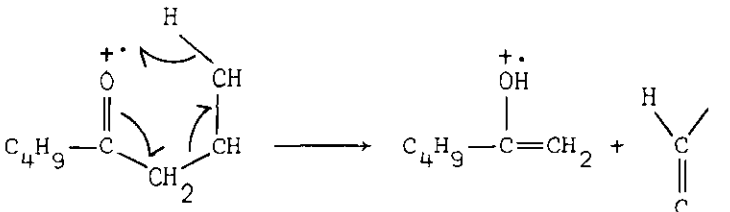
Another point to be noted in the low mass region is the absence of McLafferty rearrangement ions of the type $[CH_3O\overset{OH}{\overset{|}{C}}=CH_2-R]^{+\cdot}$. This indicates possibly that no γ-hydrogens are present for rearrangement. A possible structure conforming to this requirement would then be XXVI.



XXVI

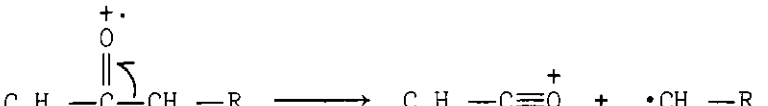
The part structure ($-CH_2CO_2CH_3$) must be present to account for the loss of 73 from $M^{+\cdot}$.

There is, however, an abundant odd-electron ion at *m/e* 100: the only apparent rearrangement peak in the spectrum. This peak and the peak at *m/e* 85 could be accounted for quite neatly if the presence of a carbonyl function were assumed as shown in equations 13 and 14.



(13)

m/e 100



(14)

m/e 85

However, it would be expected that any carbonyl formed would be further oxidized to the carboxylic acid under the reaction conditions employed.

Because of the complexity of the spectrum, then, a second oxidation was carried out, and the resulting product mixture was treated with bis-(N,O)-trimethylsilylacetamide in order to convert any acids present to their trimethylsilyl esters. From the reaction, only four products were observed by GC: once again there was one major peak constituting *ca.* 70% of the total peak area. The four spectra are given as Plates 9, 10, 11, and 12.

The first GC peak observed (peak A, Plate 9) has an ion at highest mass of *m/e* 261. The presence in the low mass ion region of *m/e* 73 (XXVII) and *m/e* 75 (XXVIII) indicate that a trimethylsilyl derivative is present.

XXVII, *m/e* 73

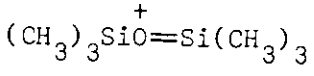
XXVIII, *m/e* 75

Since the loss of methyl (M-15) from the trimethylsilyl group is a common occurrence, it might be concluded that the molecular ion is *m/e* 276. However, the base peak in the spectrum occurs at *m/e* 173, and is very likely formed by direct cleavage. This is not observed in di-TMS esters, and it is highly unlikely that an alcohol function would have survived the nitric acid oxidation to become trimethylsilylated.

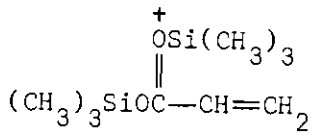
It is necessary to observe whether, in fact, there is any evidence that a di-TMS ester is present.

An abundant rearrangement ion at *m/e* 147 is present; this ion has been shown to have structure XXIX.[58] (This ion commonly occurs in many polysilylated compounds.) The presence of at least two trimethylsilyl groups in the molecule is then indicated.

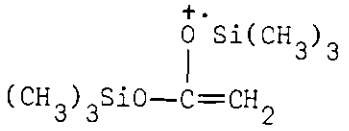
An ion is then observed at *m/e* 217 (Structure XXX). The ion could result from a rearrangement of the TMS group; several possible mechanisms[58] for formation of such ions from TMS esters of long chain dicarboxylic acids are given by Draffan, Stillwell and McCloskey.



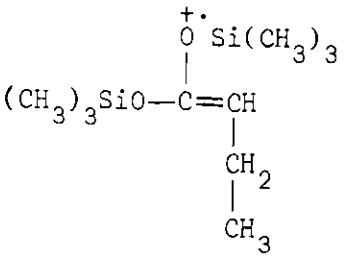
XXIX, *m/e* 147



XXX, *m/e* 217



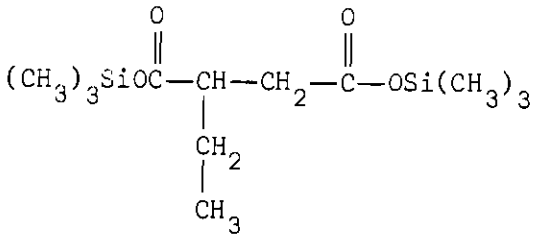
XXXI, *m/e* 204



XXXII, *m/e* 232

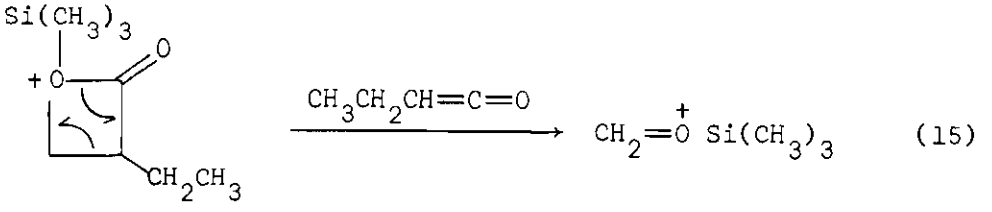
However, no ion is observed at *m/e* 204 (XXXI) which is a characteristic rearrangement ion of dicarboxylic TMS esters. An ion is, however, observed at *m/e* 232, which is a homologue of *m/e* 204, and could indicate branching in the α-position (structure XXXII).

If the ion at 261 were assumed to be M-29, the molecular ion would be *m/e* 290, which would then lose 117 mass units to form *m/e* 173. The structure then indicated is XXXIII.



XXXIII

It is possible that, after loss of the ethyl group, rearrangement could then occur to give the ion at *m/e* 217. There is a small ion present at *m/e* 103; the ion at 173 could decompose via the pathway indicated to give this ion (Equation 15). (A similar process occurs in the mass spectrum of 3-phenoxypropionic acid TMS ester.[59])



m/e 173 *m/e* 103 (15)

This interpretation is by no means conclusive, however.

The second mass spectrum observed (peak B, Plate 10) is obviously a trimethylsilyl derivative (*m/e* 73 and 75), but has no characteristic

rearrangement ions containing more than one trimethylsilyl group (*m/e* 204, 217). Hence, it is probable that a dicarboxylic acid TMS ester is not present.

The peak at highest mass occurs at *m/e* 257. It is obvious from the isotope ratios that the ion must have the formula $[C_{14}H_{29}SiO_2]^+$ or $[C_{13}H_{27}SiO_3]^+$. Since an abundant ion of (M-15) usually occurs in TMS ester spectra (in some compounds this ion is the base peak), the parent ion may be considered to be *m/e* 272. The carboxylic acid corresponding to this TMS ester would then have the formulas $C_{10}H_{18}O_2$ or $C_9H_{14}O_3$.

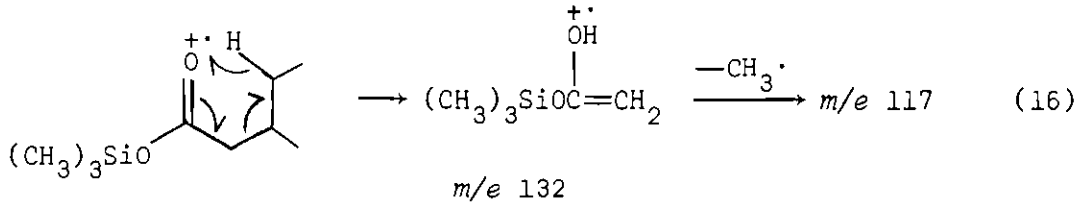
It is obvious by simple arithmetic that *m/e* 272 cannot be a di-TMS ester of a dicarboxylic acid, as (272-117-117) would give 38 mass units: this does not correspond to any saturated combination of CH or CHO.

If *m/e* 272 is the molecular ion, it could then lose 59 mass units to form the abundant ion at *m/e* 213. Teeter[60] has shown that in trimethylsilyl benzoate, an abundant ion at M-59 is due to rearrangement of the ion at M-15 followed by expulsion of carbon dioxide.

Naturally, for a similar transition to proceed in the observed molecule, a site must be present to which the silyl group could be rearranged: this requires the presence of at least one oxygen atom. In the lower mass ion region, the series (45,59,...) is observed, also indicating oxygen.

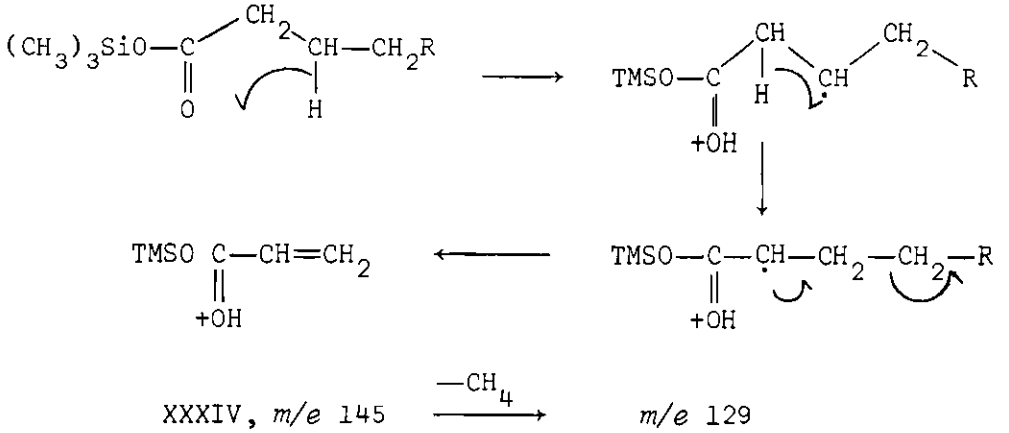
There are two types of ions produced in dicarboxylic acid TMS esters that could also be produced in monocarboxylic esters, and these are considered below.

A typical McLafferty rearrangement (Equation 16) produces an ion as shown below. This ion then loses methyl from the trimethylsilyl moiety to form *m/e* 117.



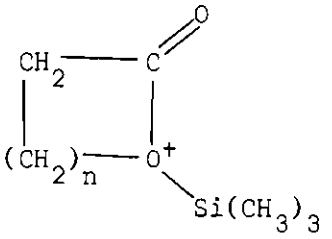
m/e 132 (16)

A second type of rearrangement occurs analogously to that observed for methyl esters to give the ion series (87,143,199,255,...) spaced 56 mass units apart.[61] In this rearrangement a hydrogen is abstracted from a point on the chain as shown in Scheme 3.



XXXIV, *m/e* 145 $\xrightarrow{-CH_4}$ *m/e* 129

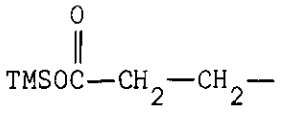
The ion at *m/e* 145 loses CH_4 easily to form *m/e* 129. It is to be noted that ions of structure XXXIV are isobaric with those observed from direct cleavage. Stable ions resulting from direct cleavage might be expected to have the cyclic structure XXXV.



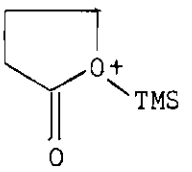
XXXV

In the spectrum of peak B, an ion is observed at *m/e* 132. Ions are also observed at *m/e* 145 and 129, indicating a transition of the type outlined in Scheme 3. A conclusion may be made that the part structure XXXVI is present.

A large peak is observed at *m/e* 159. This ion must then result from direct cleavage and have the structure XXXVII.



XXXVI

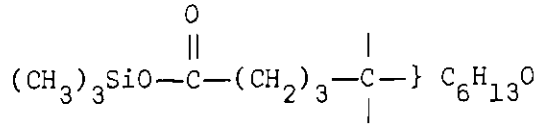


XXXVII

This is a very stable ion.[59]

The base peak in the spectrum is observed at *m/e* 157. This ion is 56 mass units ($4CH_2$) less than the ion at *m/e* 213. Possibly this latter ion decomposes to give the ion at *m/e* 157.

However, the only conclusions to be drawn at present is that the molecule possibly has the part structure XXXVIII.



XXXVIII

It should be remembered that this is possibly not a homogeneous GC peak, also.

The third GC peak to consider (peak C, Plate 11) has its ion at highest mass occurring at *m/e* 284. This is a good choice for the molecular ion, since the ion can then lose methyl to form the abundant ion at *m/e* 269, and 59 mass units, as previously outlined for peak B, to form the ion at *m/e* 225.

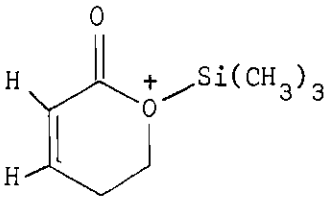
This transition again would require the presence of an oxygen atom in the residue to which the TMS group could be rearranged.

The most striking feature of the spectrum is the loss of 43, 57, 71, 85, 99, 113, 127, and 143 mass units to form the silicon-containing ions at *m/e* 241, 227, 213, 199, 185, 171, 157, and 141. The base peak occurs at *m/e* 171 (M-113). On first glance, this would seem to indicate a long-chain mono-TMS ester, but the formulas possible for *m/e* 284 ($C_{10}H_{15}O_2CO_2TMS$, $C_{11}H_{19}OCO_2TMS$, $C_{12}H_{23}CO_2TMS$) indicate at least one ring

or double bond in addition to the carboxyl group. The first formula given is too highly unsaturated and the third given contains no rearrangement center; therefore the second formula is the formula of choice. It is to be noted that an ion occurs at (M-117), at *m/e* 167. This ion, if it is represented by the formula $[C_{11}H_{19}O)^+$, must contain two rings.

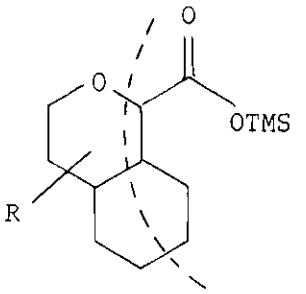
Thus a possible picture emerges of a parent molecule having two rings and an oxygen atom suitably located for transfer of a trimethylsilyl group.

The question arises as to whether the ions at mass 171, 185, 199, ..., etc. contain the carboxysilyl group or whether they are due to a rearranged molecule that has eliminated the carbonyl group. It is not possible to say for certain without high resolution measurements. It might be said, however, that structure XXXIX would be expected to be a very stable ion, possibly eliminating methyl to give the abundant ion at *m/e* 156.



XXXIV, *m/e* 171

It is possible that such an ion could arise from a structure such as XL.



XL

Without further information, no other conclusions can be drawn from this spectrum.*

The last TMS ester to be considered is the largest GC peak. The base peak in the spectrum is at *m/e* 374, which may also be the molecular ion. A loss of (M - 15) gives *m/e* 359, while loss of 59 mass units occurs to give *m/e* 315. A peak at 341 corresponds to loss of 18 mass units (H_2O?) from *m/e* 359. An inspection of the lower mass ion region indicates that a di-TMS compound is present. An abundant ion is observed at *m/e* 204 (structure XXXI).

An abundant ion is also observed at *m/e* 147 (structure XXIX).

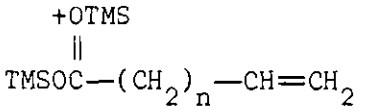
---

*The TMS ester represented by this spectrum appears to correspond to the methyl ester represented by the spectrum of peak No. 2 in Table 10. The molecular ion in the latter is at *m/e* 226; a calculation will show that (226 - 59 + 117 = 284). The material also exhibits an abundant ion at *m/e* 167. However, an abundant odd-electron ion appears at *m/e* 158, which could possibly correspond to

$$[CH_3OC(OH)=CH-C_6H_{13}]^{+\cdot}$$

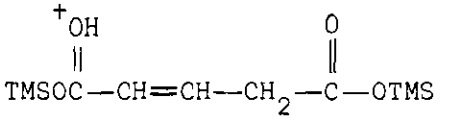
This is at variance with the conclusions drawn above for the TMS ester.

An ion is observed at *m/e* 217 (structure XXX); possible homologues of this ion occur at *m/e* 231, 245, and 259. These ions are given the structure XLI.[58]

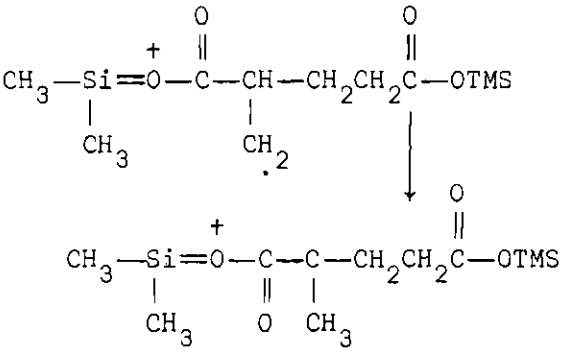


XLI

A pair of ions occurs at *m/e* 275 and *m/e* 274. This corresponds to a loss of 99 and 100 mass units from the molecular ion. The ion at *m/e* 274 could also arise by loss of 85 mass units from *m/e* 359. It is possible that, in the absence of silyl rearrangements, these transitions could result from loss of an alkyl or cyclic ether side chain (85 = C_6H_{13} or C_5H_9O), giving the structures XLII and XLIII for the ions at *m/e* 274 and 275, respectively.



XLII, *m/e* 275



XLIII, *m/e* 274

For the loss to 274 to occur, a point of facile cleavage must be present. It is also to be noted that the ion at *m/e* 275 can then lose CH_4 as previously indicated in Scheme 3 to form the ion at *m/e* 259. However, this latter ion is not very abundant.

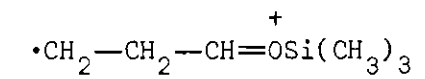
The ion at *m/e* 233 corresponds to loss of 141 mass units from the molecular ion. This could possibly be loss of the complete alkyl or alkyloxy residue, plus carbonyl, to give an ion having the formula $(TMS)_2O_3C_3H_3$.

There are abundant peaks at *m/e* 227 and 225, which correspond to loss of 147 and 149, respectively, from the molecular ion.

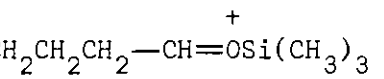
The ion at *m/e* 189 does not seem to correspond to any well-known TMS ester cleavage products. One would think it might result from the loss of CH_4 from *m/e* 204, but this transition has not been reported previously.

A very abundant ion at *m/e* 173, together with an abundant ion at *m/e* 157, lead to the same conclusion reached for peak A (see Equation 13).

The two abundant odd-electron ions at *m/e* 130 and 144 could have structures XLIV and XLV, or an appropriate isomer.



XLIV, *m/e* 130



XLV, *m/e* 144

From a consideration of the fragmentation peaks, it is very difficult to ascertain any structure for the parent molecule.

A problem also arises when comparing the spectrum of this TMS ester of the nitric acid oxidation product with the spectrum of its corresponding methyl ester from a previous reaction. If the molecular weight of this di-TMS ester is indeed 374, then the molecular weight of the corresponding dimethyl ester would be (374 - 117 - 117 + 59 + 59 = 258). However, in the spectrum of the dimethyl ester, the peak observed at highest mass was at *m/e* 255, leading to the conclusion that the molecular ion must be 286.

Working backwards, then, the di-TMS ester corresponding to this dimethyl ester would have a molecular weight of 402. This would then mean, that, in the fragmentation of this ester, 28 mass units (CO or $CH_2{=}CH_2$) would be lost in such a facile process that it would suppress the normal observation of the molecular ion and the M-15 peak. This is heretofore unknown in the spectra of TMS esters.

This, then, is yet another anomaly encountered in the structure elucidation of compound X.

It was thought that perhaps permanganate oxidation of didehydro X would yield products that would be helpful in elucidating the structure of the didehydro compound. Oxidation was carried out using 2% aqueous potassium permanganate; since didehydro X was insoluble in water, acetone was used as the solvent.

The *p*H of the resulting solution was adjusted to 1.5 and the solution was steam distilled to remove volatile acids. The *p*H of the distillate was adjusted to 11, the solution was evaporated and the residue was treated with *p*-phenylphenacyl bromide to convert any

volatile acids present to their *p*-phenylphenacyl esters. Analysis of the resulting products by comparison of retention times with those of standard *p*-phenylphenacyl esters indicated that *p*-phenylphenacyl acetate and propionate were present.

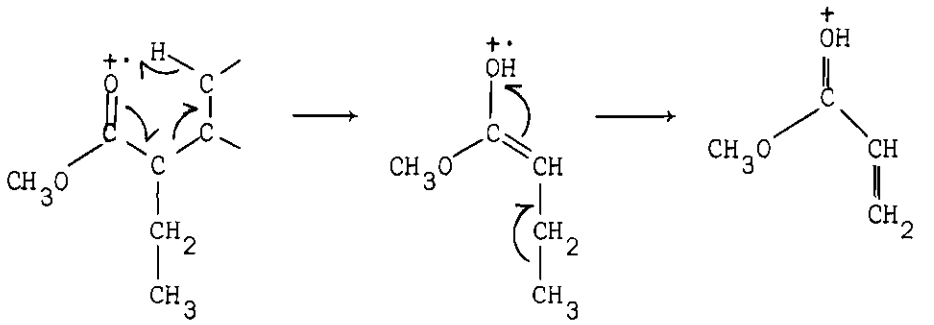
The residue remaining after steam distillation was dissolved in water and extracted with chloroform. Chloroform was evaporated from the extract to yield a residue, which was then treated with 2,2-dimethoxypropane and hydrochloric acid to convert any acids present to their methyl esters. The product mixture was then subjected to GC-MS analysis; the results are given as Table 12.

The spectrum of the GC peak observed contains no abundant ion at *m/e* 59; the ion observed at highest mass is *m/e* 126. Losses of (M-24) and (M-27) to the next most abundant ions (*m/e* 102 and *m/e* 99) make it unlikely that *m/e* 126 is the molecular ion. It is also improbable that a methyl ester is present.

In addition, the retention times of the peaks were compared with retention times of standard dicarboxylic acid methyl esters to ascertain the approximate molecular weight. The retention time of the first peak was in the region of that observed for dimethyl adipate (mw 174). A cyclic or branched molecule, of course, would have to have a higher molecular weight than dimethyl adipate in order to be observed at the same approximate retention time. This further indicates that *m/e* 126 cannot be the molecular ion.

The next GC peak observed, peak No. 2, appears to have no molecular ion as well. However, the base peak occurs at *m/e* 102, and an ion,

albeit small, occurs at *m/e* 59. An α-ethyl-substituted methyl ester would give *m/e* 102 as a result of McLafferty rearrangement[62] as shown in Equation 17.

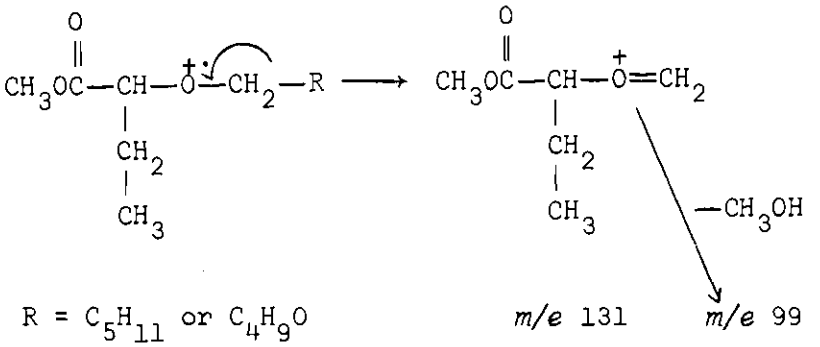


m/e 102 *m/e* 87 (17)

The lack of a molecular ion peak makes further interpretation of the spectrum difficult. It may be observed, however, that there are two peaks of low abundance at *m/e* 143 and *m/e* 171. If one considers 202 as the molecular ion, then losses of (M-15) to give *m/e* 171 and (M-59) to give *m/e* 143 would occur. A loss of (M-71) would give the ion at *m/e* 131.

It might also be noted that the GC peak observed had a retention time comparable with that of 2,5-dimethyladipic acid (mw 202).

The ion at *m/e* 131 might easily be explained by an ether function in the molecule, as shown in Equation 18.



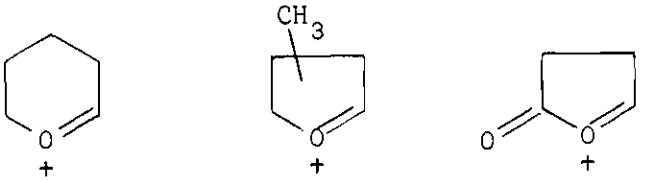
(18)

R = C_5H_{11} or C_4H_9O *m/e* 131 *m/e* 99

The third GC peak to be considered has abundant ions at *m/e* 56, 99, and 113. This is essentially the same spectrum that was observed for a product of sequential reductions (!) (see Table 5). At present, the only additional comment to be made is that from GC retention times, this compound (or compounds) must have a molecular weight in the region of a C_8-C_{10} dicarboxylic acid methyl ester.

The fourth GC peak gives no molecular ion, and furthermore, does not exhibit an ion at *m/e* 59. The base peak is observed at *m/e* 85; this cleavage is highly predominant, as this ion carries 23% of the total ion current.

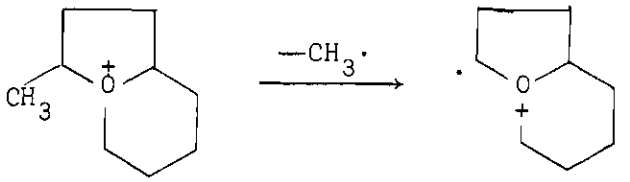
Molecules giving this typical abundant *m/e* 85 ion are tetrahydropyrans,[63] methyltetrahydrofurans,[64] and γ-lactones[48,65] (XLVI, XLVII, and XLVIII).



XLVI XLVII XLVIII

The interesting ion *m/e* 72 is present; however, without knowledge of the molecular ion, it is difficult to speculate upon its genesis.

The ion present at highest mass is *m/e* 141. This is *m/e* 85 + 56 mass units, and could indicate a structure such as XLIX, which could then lose methyl to form the ion at *m/e* 126 as shown in Equation 19.



(19)

XLIX, *m/e* 141 *m/e* 126

From GC retention times, this compound evidently has a molecular weight somewhere in the C_{11}-C_{13} dicarboxylic acid methyl ester region. However, since no ion at *m/e* 59 is present, this compound is probably not a methyl ester. The reaction conditions and procedures for work-up allow the presence of neutral compounds: it is possible that a neutral compound in the weight range indicated above is present.

However, without further knowledge of the compound and/or high resolution data, the fragmentation patterns do not give sufficient information to determine the structure of this compound.

CHAPTER IV

CONCLUSIONS AND RECOMMENDATIONS

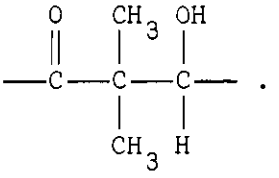
The broad-spectrum antibiotic was found to have the formula $C_{41}H_{60}N_2O_{12}$, with a molecular weight of 773. The neutralization equivalent indicated that the compound was monoacidic; the saponification equivalent indicated two groups that were labile to base. Depending upon whether the acidic function was carboxylic or phenolic, the molecule could have a maximum of six or seven hydroxyl groups, respectively. A mass spectrum of a trimethylsilyl ether derivative of compound X indicated that six hydroxyl groups were possible. The molecule was also found to have eight active hydrogens, due to the hydroxyl group and the acidic function, plus other functions, possibly active methylene or amide.

The molecule was found by analysis to have two *N*-methyl groups; this finding was substantiated by the finding of methylamine among products of acidic and basic hydrolysis.

The one *O*-methyl group indicated by analysis was also indicated by the nmr spectrum of the molecule. The nmr also demonstrated the presence of at least four *C*-methyl groups; analytical data (Kuhn-Roth (7.05) confirmed this finding, suggesting four or more *C*-methyl groups.

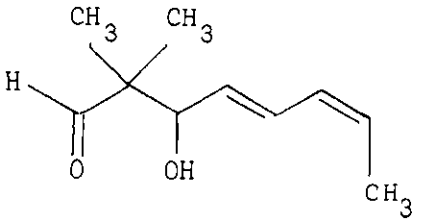
Both the yellow color of the antibiotic and its uv absorption indicated conjugated systems. Approximately 15 protons bonded to olefinic groups were observed in the nmr spectrum.

Isobutyraldehyde was isolated from a basic hydrolysis, suggesting the grouping

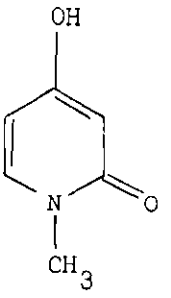


The isobutyraldehyde possibly arose via reverse aldol condensation.

2,2-Dimethyl-3-hydroxyocta-4,6-dienal (L) was isolated by Hoffman-LaRoche Inc. from products obtained from a periodate oxidation of X-5108.[1] 1-Methyl-4-hydroxy-2-pyridone (LI) was found to result when X-5108 was treated with boiling water.



L



LI

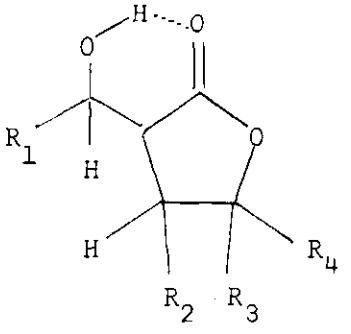
It is possible that isobutyraldehyde could have been derived from the structural moiety represented by structure L Structure LI may form part of the uv chromophore. The methylamine found as a result of hydrolysis could have come from this part of the molecule.

A fragment having the formula $C_{16}H_{28}O_5$ was isolated in these laboratories from products of hydrogenation of X-5108. The formula was

confirmed by high-resolution mass spectrometry; the formula indicates that three rings and/or double bonds must be present in the molecule. The purified white, crystalline material was found to be homogeneous by thin-layer chromatography.

Hydroxyl groups and a γ-lactone function were indicated by the ir spectrum. The saponification equivalent indicated one group labile to base; the uv spectrum was consistent with the presence of a lactone function. The lactone ring would account for two of the rings-plus-double bonds.

A total of two hydroxyl groups, one probably hydrogen-bonded, was indicated by the nmr spectrum. The presence of a monodehydro X suggests that one hydroxyl group is lost more readily than the other. The uv and ir spectra of didehydro X indicates that an α,β-unsaturated γ-lactone functionality could be present. Hence, if the hydroxyl group is placed in a suitable position for hydrogen bonding, the following part structure (LII) would result.



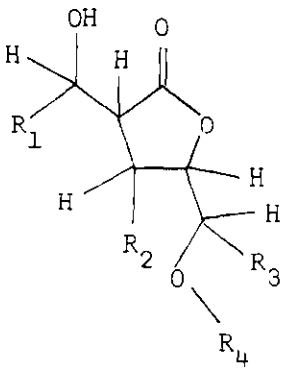
LII

This structure would account for the presence of a sharp triplet at τ 7.42 in the nmr spectrum of compound X (deuterochloroform

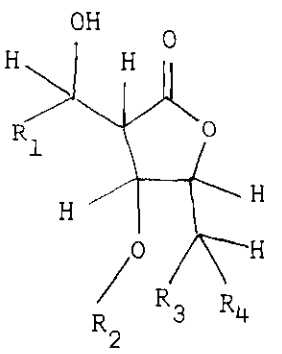
solution). The proton giving rise to this triplet was found to exchange with deuterium in D_2O-pyridine-d_5 solution. A model β-hydroxy ester failed to show exchange; it is recommended that better model compounds, especially γ-lactones, be studied.

The presence of a hydrogen atom at R_3 or R_4 of LII is indicated by the nmr absorption at τ 5.60. The absorption in the similar position in butyrolactone occurs at τ 5.63.[66]

Spin-decoupling experiments in pyridine-d_5 and pyridine-d_5-deuterium oxide solutions indicate that this proton (shifted from τ 5.60 in deuterochloroform to τ 5.10 in pyridine-d_5) is coupled to another proton (shifted from τ 6.35 in deuterochloroform to τ 5.89 in pyridine-d_5 attached to a carbon bearing oxygen. Consequently, the part structure LIII or LIV is indicated.



LIII



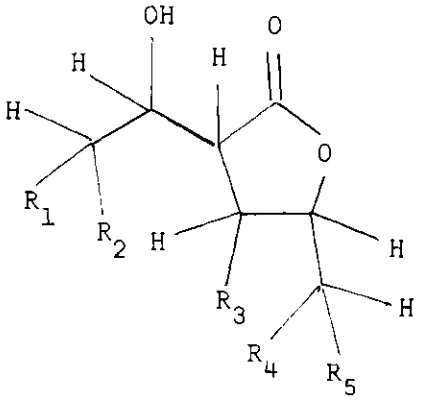
LIV

The presence of a four-line absorption for each of the low-field protons just mentioned makes it possible that four protons exist bonded to four adjacent carbon atoms as shown in structures LIII and LIV.

These structures have a total of $C_6H_6O_4$, leaving a total of $C_{10}H_{16}O$ to be placed in the molecule. Of these, C_2H_6 will account for

two geminal or isolated (singlet nmr absorptions) methyl groups. The nmr spectrum also indicated an ethyl group attached to a carbon bearing oxygen (see structure VIII). This leaves a total of ($C_{10}H_{16}O$ - C_2H_6 - C_2H_5 = C_6H_5O) to be placed.

Structures LIII and LIV account for all of the low field protons; therefore any further carbons bound to oxygen must not be bound to hydrogen. The presence of the broad triplet at τ 6.55 must then be accounted for by the proton attached to the carbon bearing the hydroxyl group β to the lactone carbonyl. For this requirement, the structure LV must obtain.



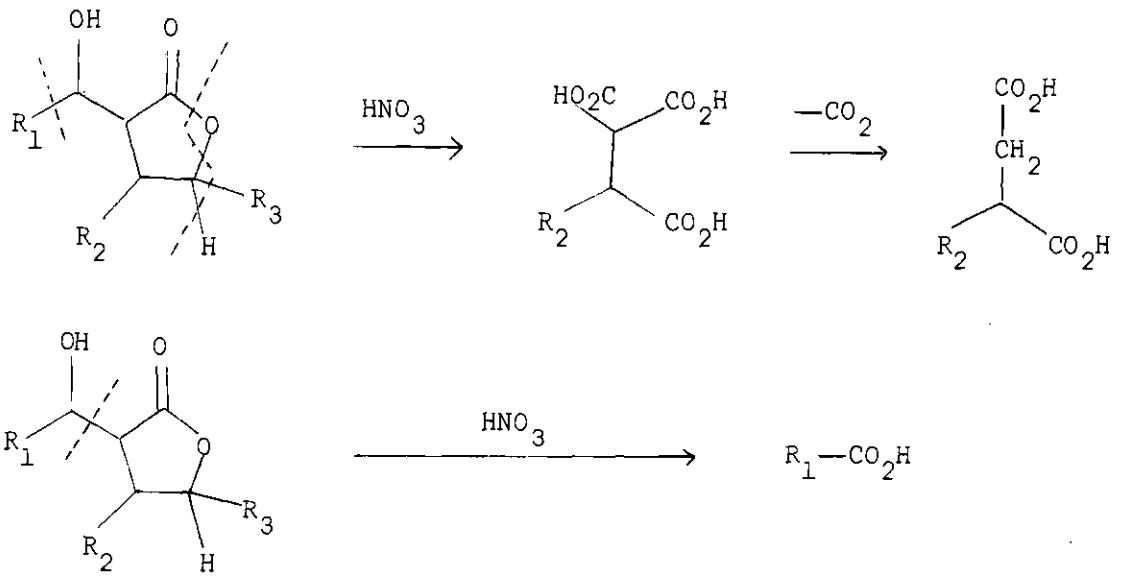
LV

Before going any further, it might be well to consider the information derived from the nitric acid oxidation. For the hydroxyl group located β to the lactone carbonyl, cleavage would take place as indicated by Scheme 4. Since a major product is obtained that has lost at most four or five carbon atoms, this would mean that R_2 must carry most of the rest of the molecule.

If, however, cleavage takes place as demonstrated in the lower half of Scheme 4, R_1 must be very large, contain another hydroxyl group,

or be attached to other portions of the molecule via a ring system. (Compound X has one more ring to be accounted for).

Scheme 4



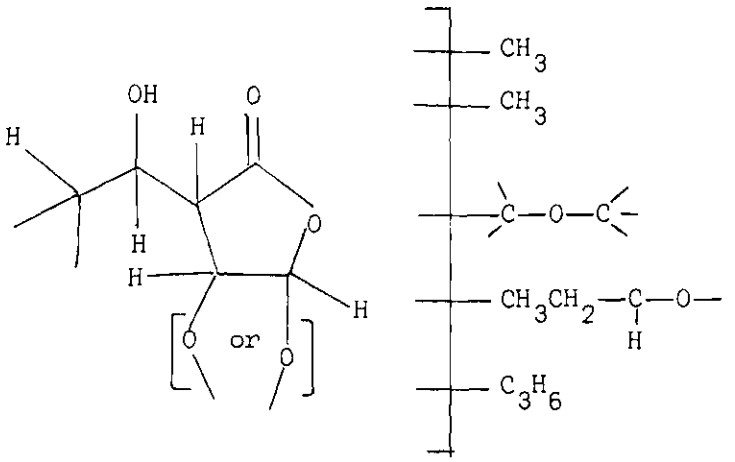
One may conclude, then, that R_3 is not large unless connected to other portions of the molecule.

It is recommended that the major nitric acid oxidation product be isolated and studied by other spectral methods; a high resolution mass spectrum of this product would be most helpful.

Compound X has been shown by nmr to have only two exchangeable protons due to hydroxyl groups. Therefore, an ether oxygen must be present to account for the remaining oxygen. The positions α to this ether must not contain additional hydrogen atoms.

It is then concluded that the structure of compound X must

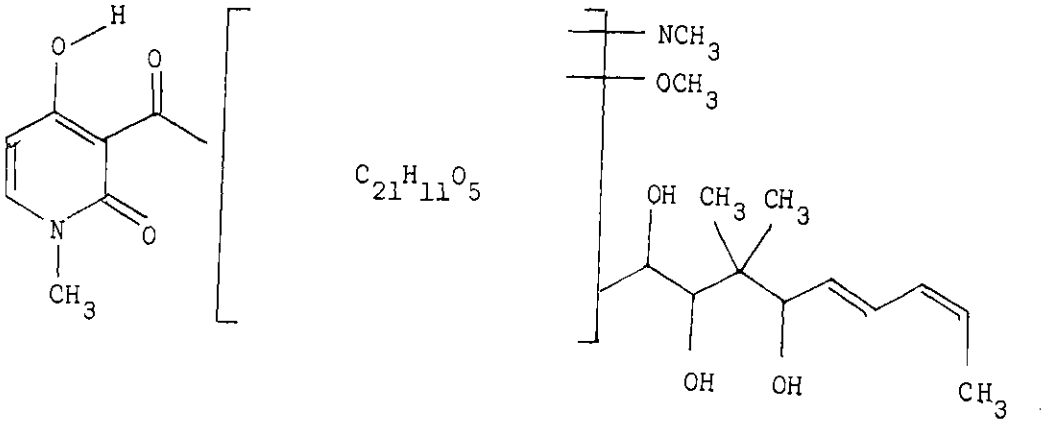
satisfy the partial structural formula LVI (some atoms may be superimposed with others).



It is recommended that didehydro X also be studied more thoroughly; this material is possibly a rearrangement product of compound X. It might also be helpful to carry out a quantitative periodate oxidation of compound X.

To summarize, it is thought that the fragments isolated by Hoffmann-LaRoche Laboratories are terminal points of the antibiotic and that the region of the molecule giving rise to compound X is located near the center. All fragments together account for 32 carbon atoms and 9 oxygen atoms, leaving only 9 carbon atoms to account for. There are also one *O*-methyl and one *N*-methyl group to be placed, and three hydroxyl groups. It has been suggested[1] that the 1-methyl-4-hydroxy-2-pyridone (LI) fragment is bonded in the antibiotic at the 3-position to a carbonyl function. The aldehyde formed (L) must of course derive from a glycol linkage. It is thought that compound X is

not present as such in the molecule, but derives from a "pre X" fragment by hydrogenolytic cleavage, hydrogenation, and possible cyclization. The above is summarized in structure LVII.



LVII

APPENDIX

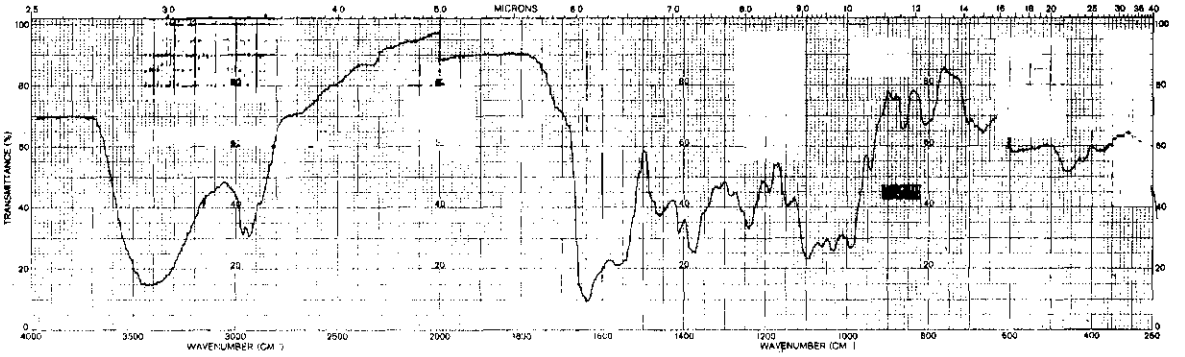


Plate 1. Infrared Spectrum of X-5108 (H^+), KBr Pellet

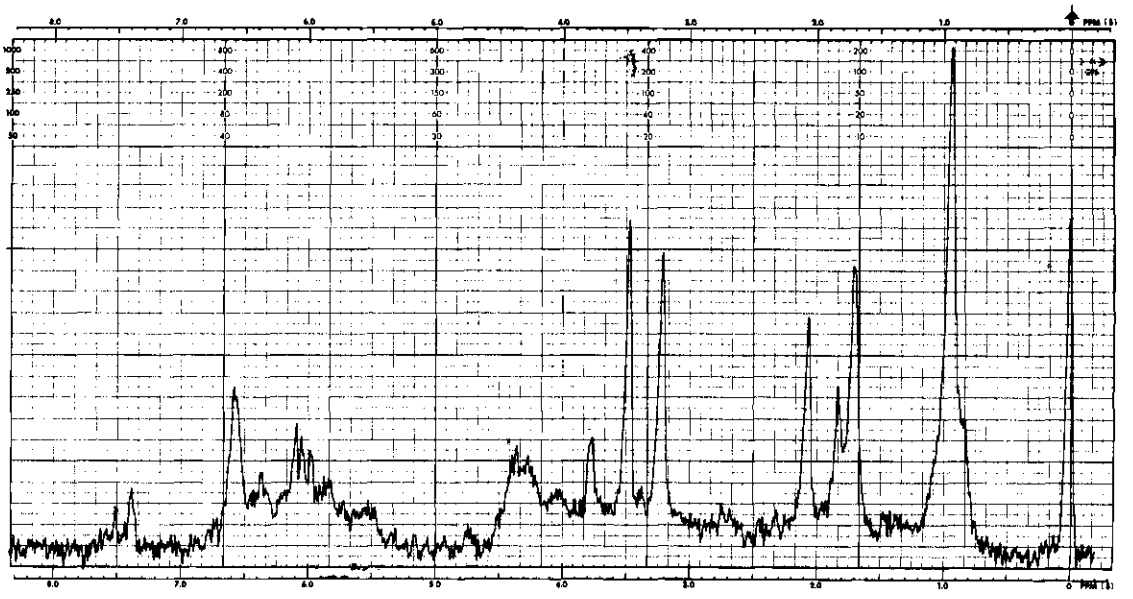


Plate 2. 60 MHz Nmr Spectrum of X-5108 (H^+) in Deuterochloroform

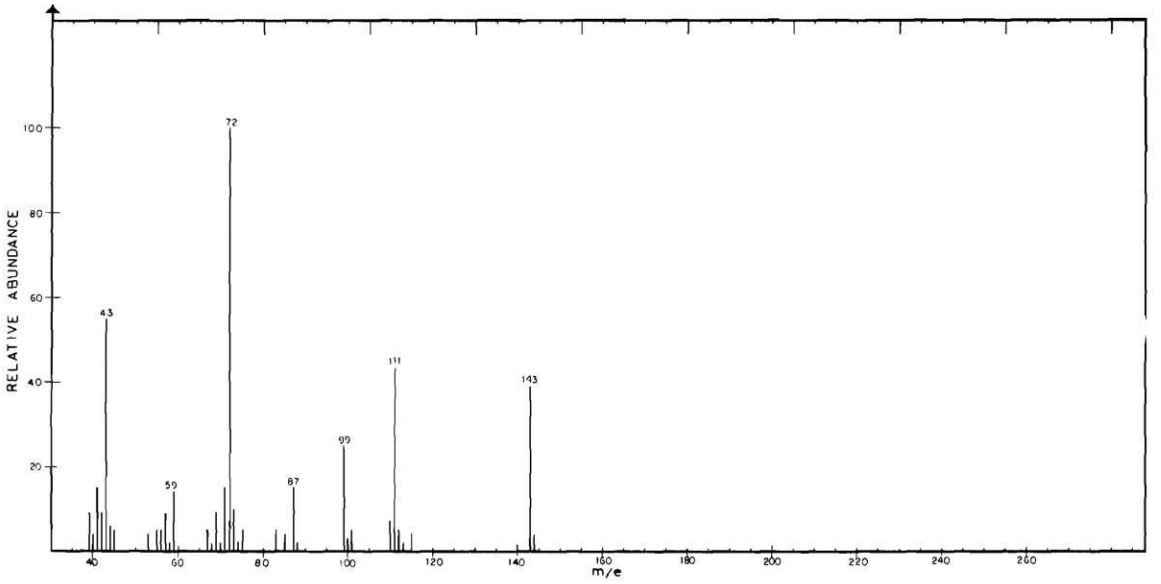


Plate 3. Mass Spectrum of the Most Intense GC Peak from Permanganate Oxidation of X-5108

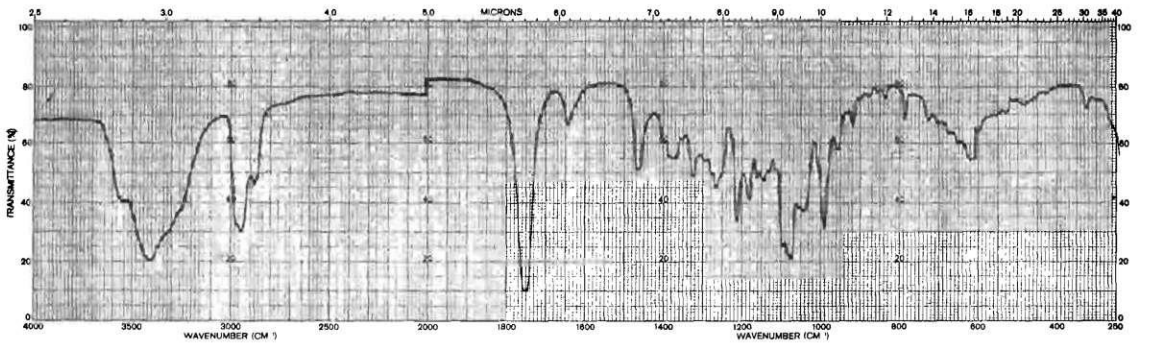


Plate 4. Infrared Spectrum of Compound X (KBr Pellet)

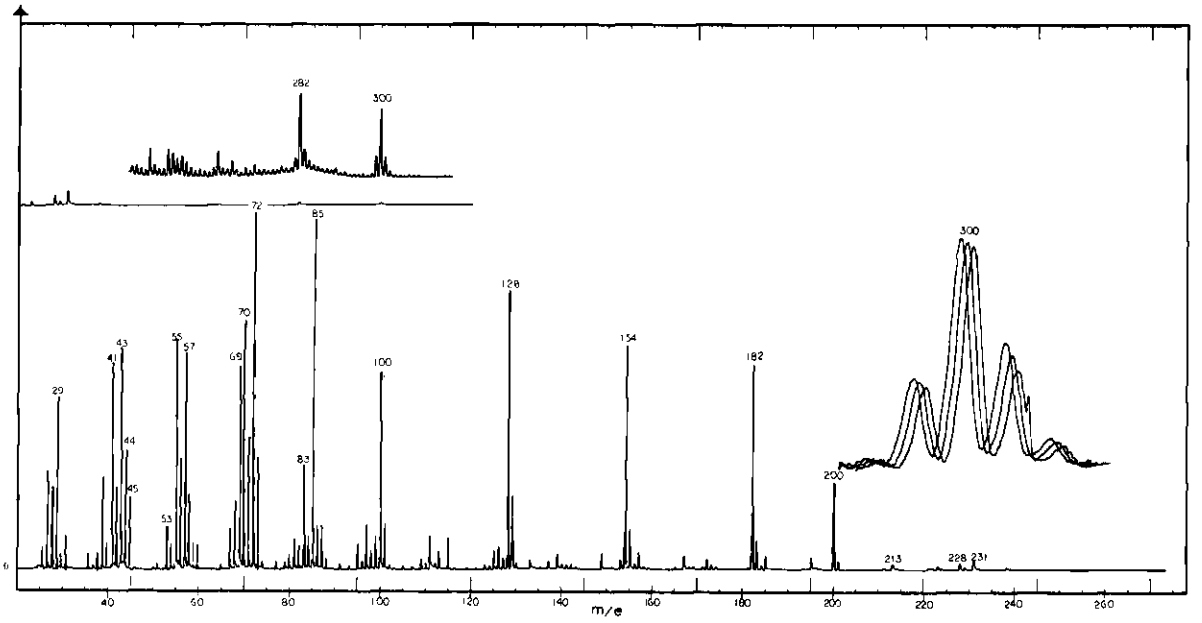


Plate 5. Low Resolution Mass Spectrum of Compound X

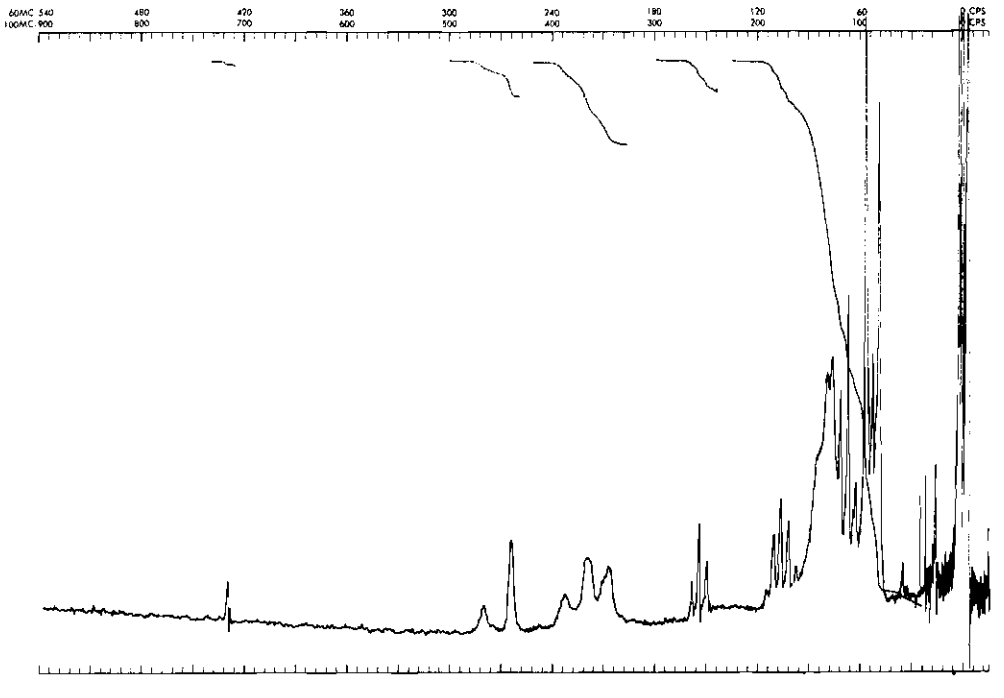


Plate 6. 100 MHz Nmr Spectrum of Compound X in Deuterochloroform

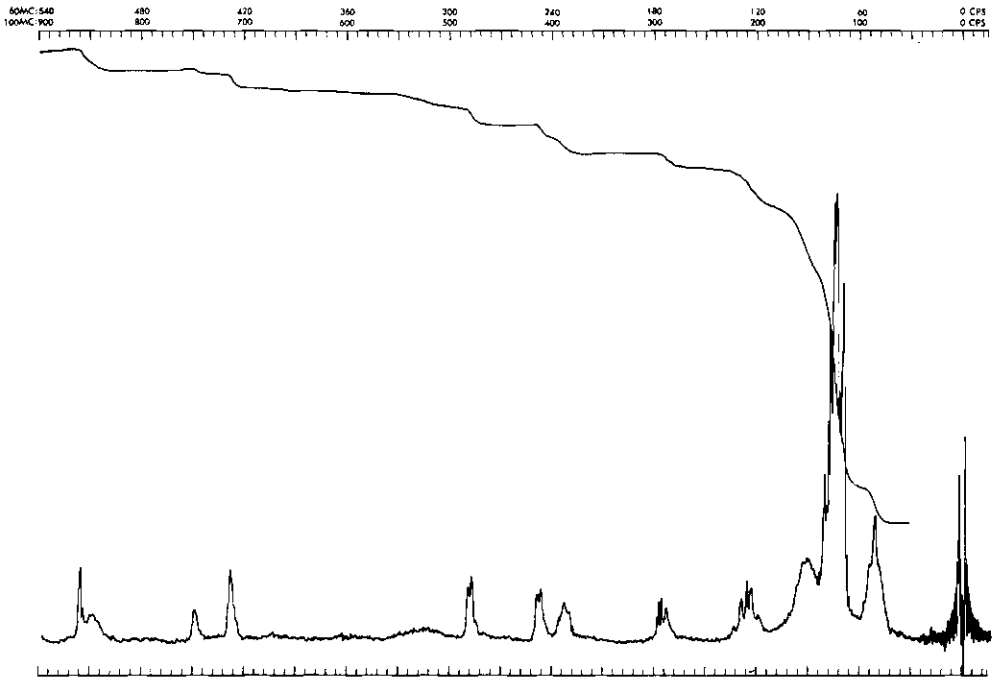


Plate 6a. 100 MHz Nmr Spectrum of Compound X in Pyridine-d_5

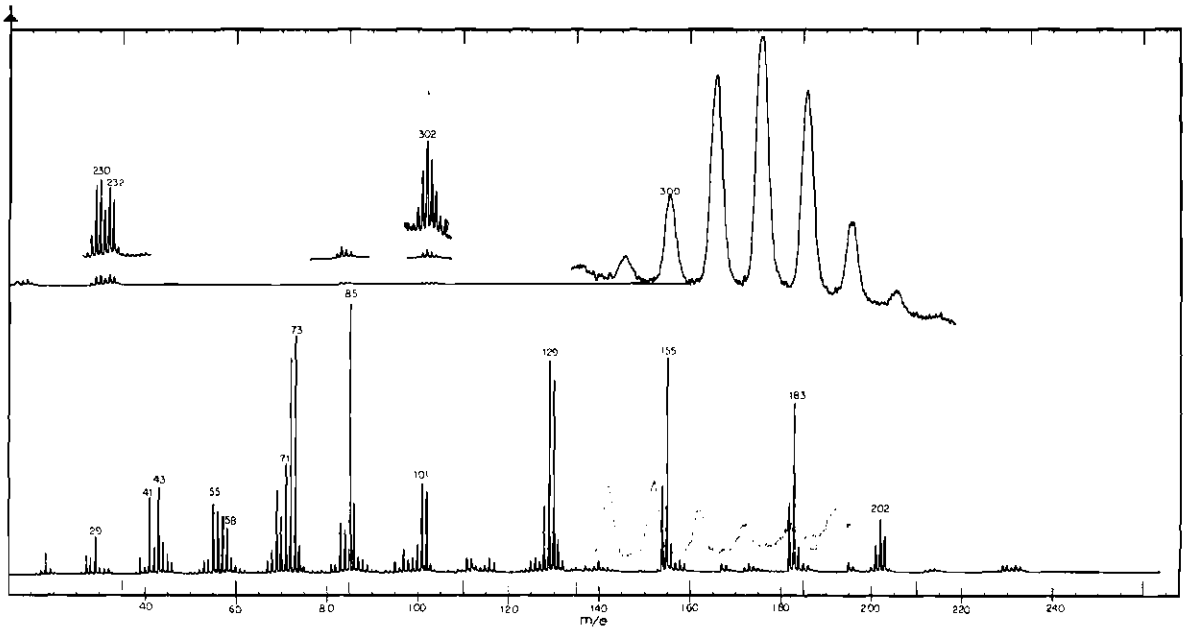


Plate 7. Mass Spectrum of Deuterated Compound X

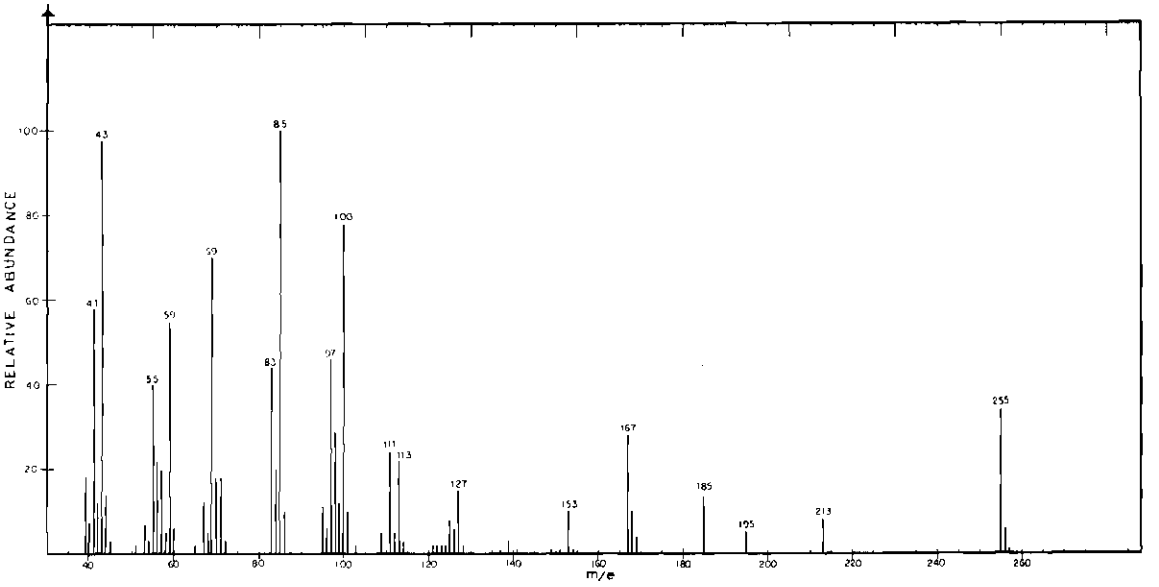


Plate 8. Mass Spectrum of Major Product from Nitric Acid Oxidation of Compound X

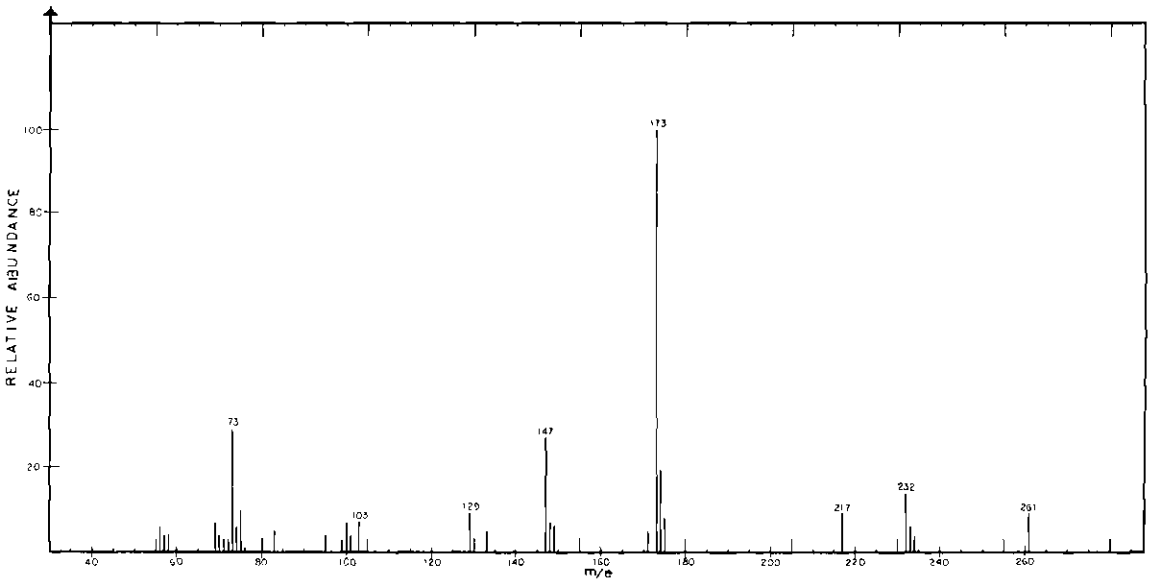


Plate 9. Mass Spectrum of Trimethylsilyl Derivative of Product from Nitric Acid Oxidation of Compound X

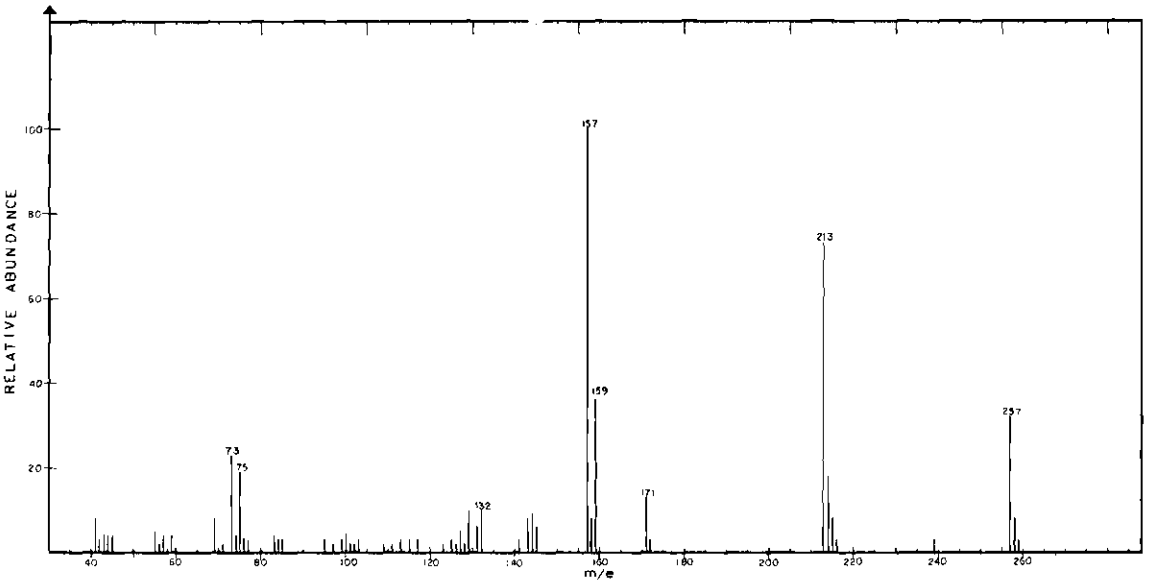


Plate 10. Mass Spectrum of Trimethylsilyl Derivative of Product from Nitric Acid Oxidation of Compound X

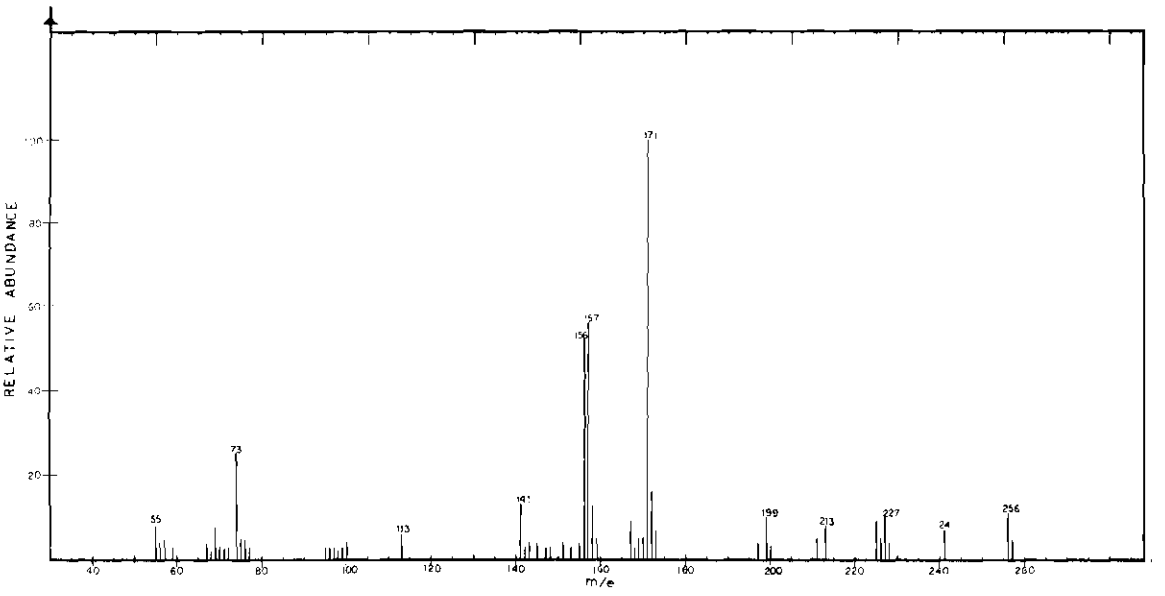


Plate 11. Mass Spectrum of Trimethylsilyl Derivative of Product from Nitric Acid Oxidation of Compound X

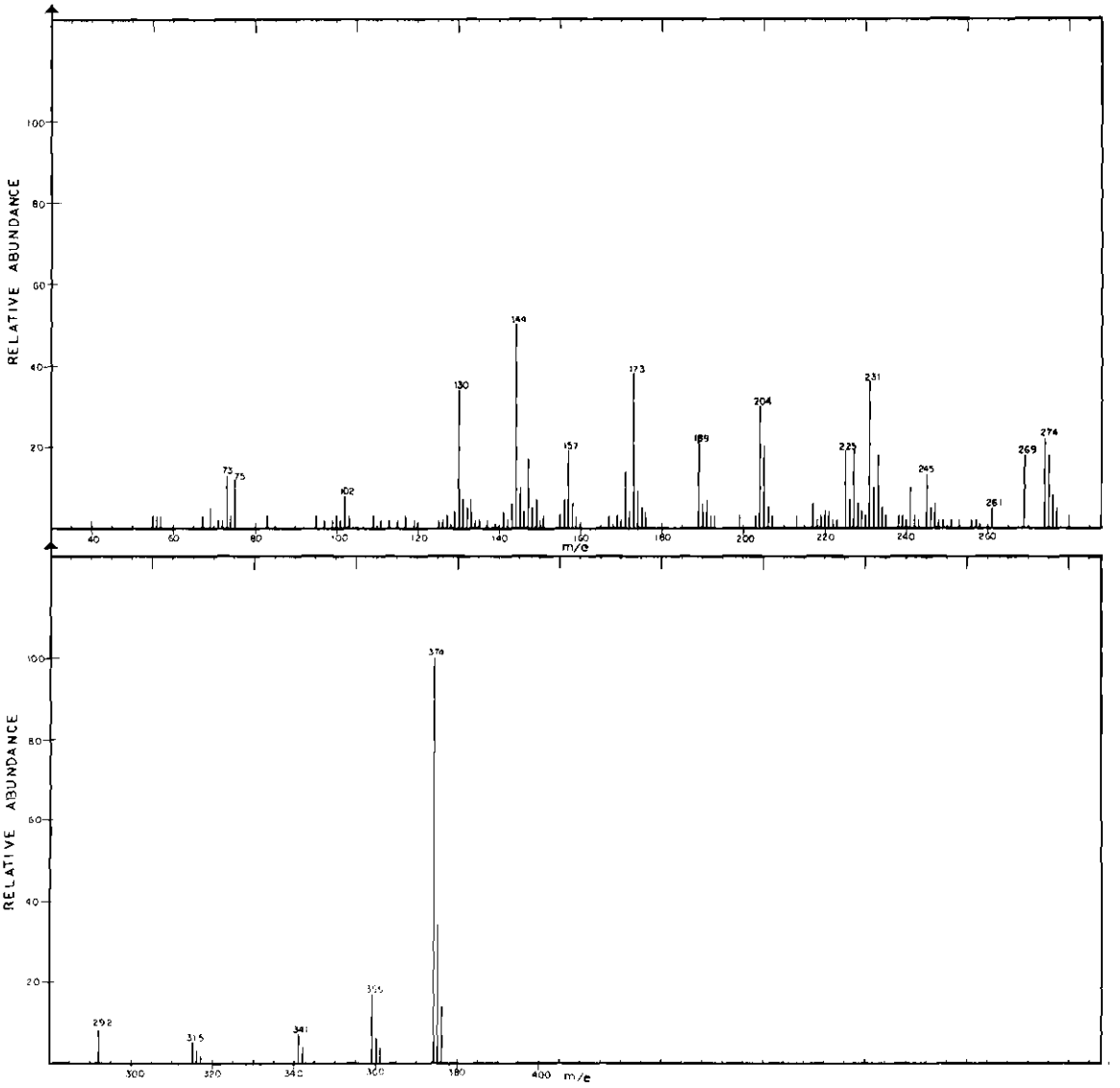


Plate 12. Mass Spectrum of Trimethylsilyl Derivative of Product from Nitric Acid Oxidation of Compound X

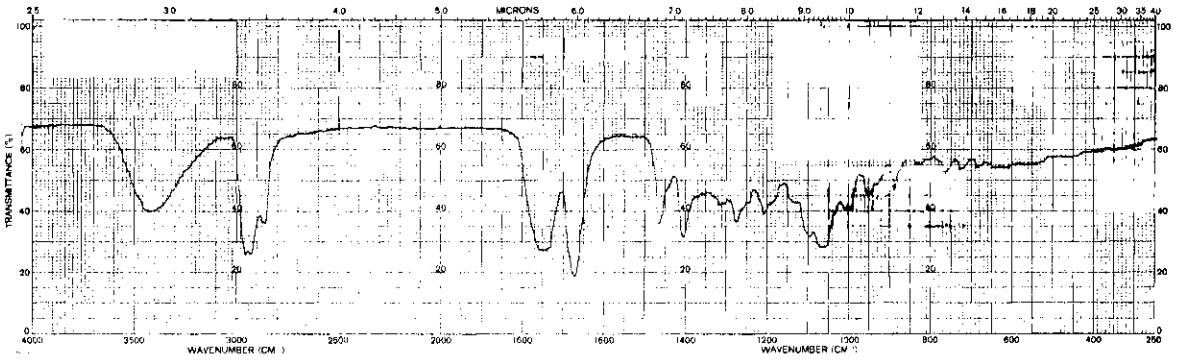


Plate 13. Infrared Spectrum of Didehydro X (Film)

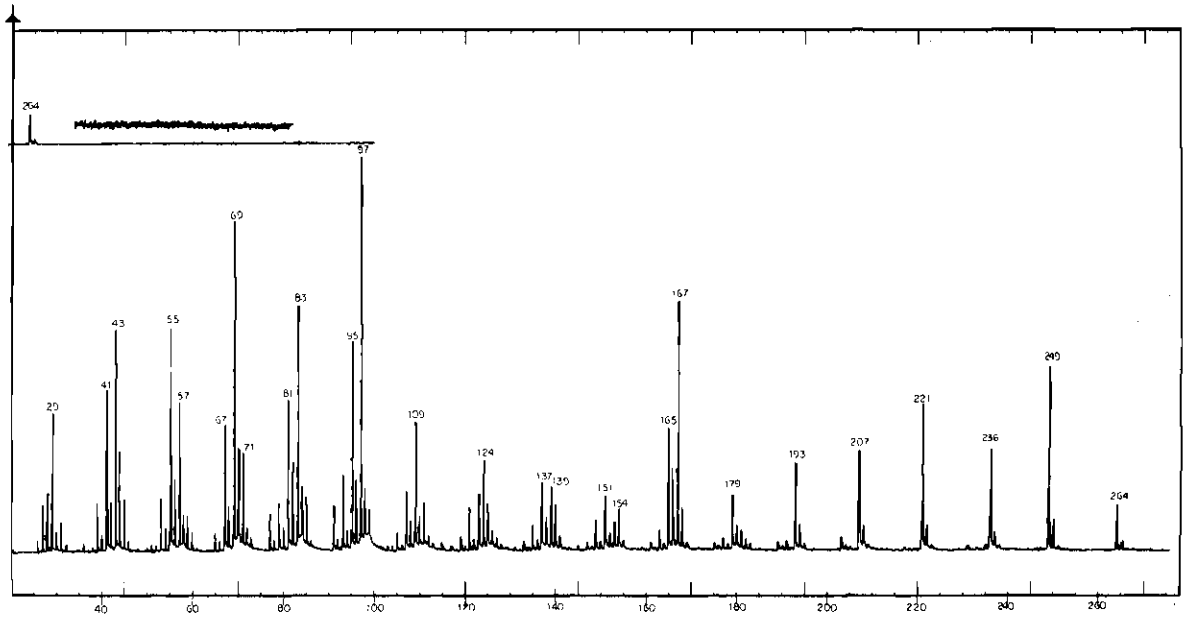


Plate 14. Mass Spectrum of Didehydro X

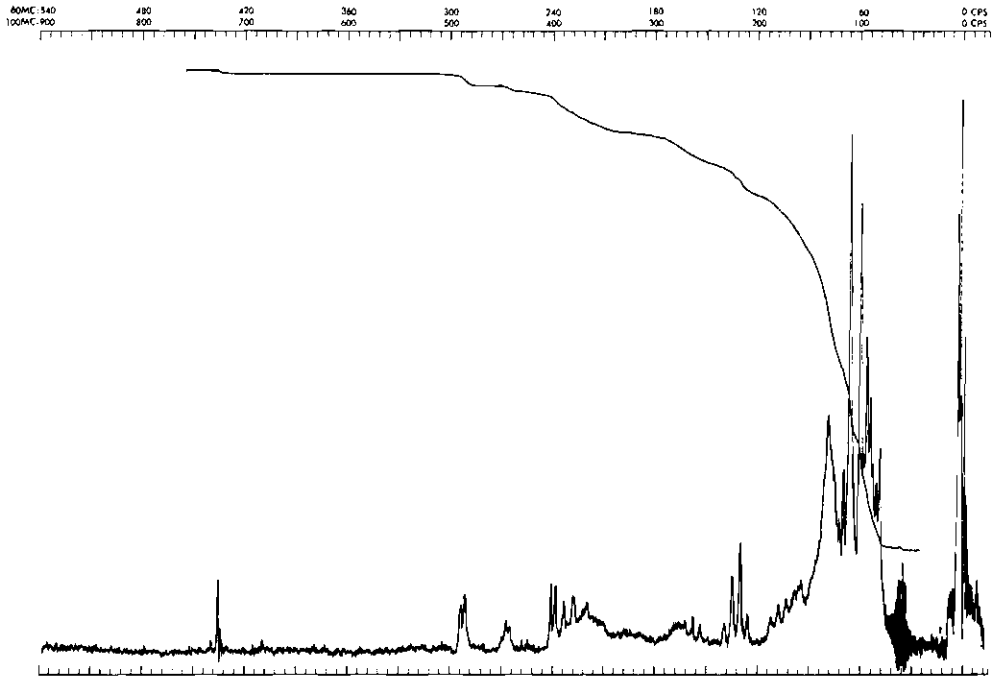


Plate 15. 100 MHz Nmr Spectrum of Didehydro X

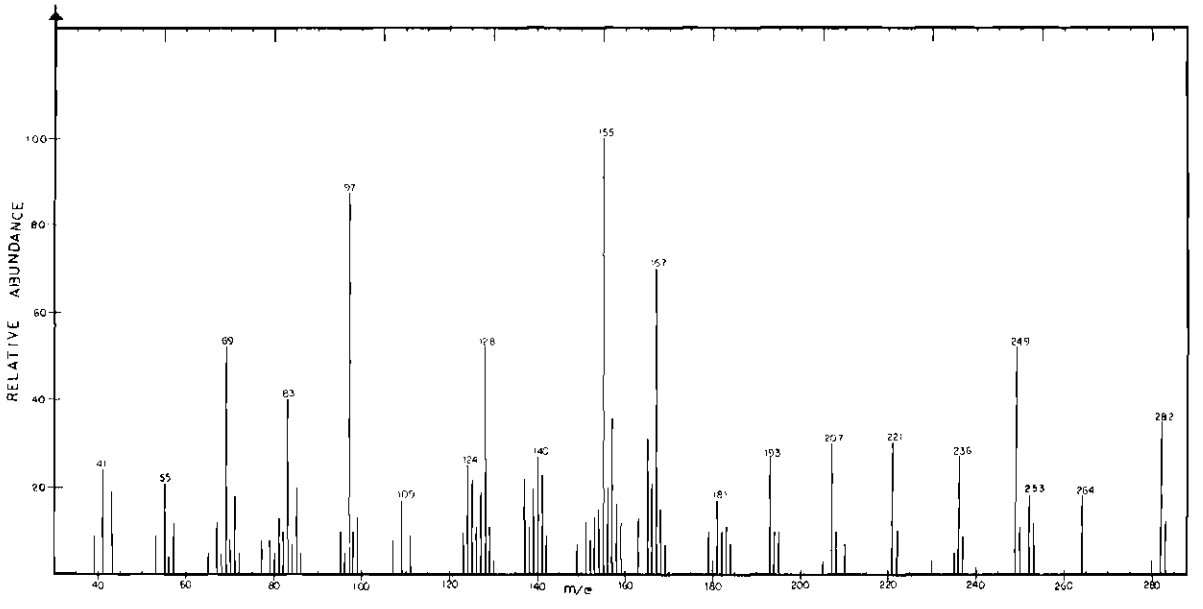


Plate 16. Mass Spectrum of Monodehydro X

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